Development of a Level 3 Wetland Assessment Template

Prepared for the Maryland Department of the Environment (MDE)
Wetlands and Waterways Program

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Introduction

The U.S. EPA defines Level 3 wetland assessment as an intensive site assessment, or a "rigorous, field-based method that provides higher resolution information on the condition of wetlands within an assessment area" (U.S. EPA, 2006). They further state that Level 3 assessment is "typically accomplished using indices of biological integrity or hydrogeomorphic function."

The Index of Biological Integrity (IBI; Karr and Chu, 1989) and similar methods (U.S. EPA, 2002a) evaluate biological condition using data on plant and animal habitat as related to anthropogenic disturbance. Sites with various levels of disturbance are compared to an undisturbed reference site, and then indicator species are used to assess condition. However, IBI's and related methods are measurements of current site condition and do not attempt to evaluate function.

The Hydrogeomorphic (HGM) approach (Smith et al., 1995) attempts to quantify wetland hydrologic, biogeochemical, and habitat functional capacity by comparing wetlands within regional subclasses to "reference standard" (least altered) wetlands of the same subclass. In the HGM approach, the *Functional Capacity Index*, or FCI, is calculated. This is defined as "an index of the capacity of a wetland to perform a function relative to other wetlands within a regional wetland subclass" in the same area.[1] Variables used to calculate an FCI almost always involve observations of biophysical structure (indicators) rather than actual measurements of functions, and in practice, sometimes the relationship of indicators to function is questionable (Cole, 2006).

One of the major assumptions made in the HGM method is that the least altered wetlands have the highest functional level, which is not necessarily true. Stander and Ehrenfeld (2009a; 2009b), in a study in northern New Jersey, found that relatively undisturbed reference wetlands did not have higher rates of nitrogen removal than highly disturbed sites. Hruby (2001) collected data along a gradient of disturbance and also reported that undisturbed wetlands in Western Washington did not have the highest level of function. The HGM approach assumes that disturbed wetlands do not have sustainable functional levels, but this was also disputed by the Washington study. Thus, we conclude that the reference standard approach tends to measure wetland condition or degree of disturbance, rather than function. That being said, we certainly agree that there is value in making both qualitative and quantitative comparisons between wetlands of interest and an appropriate reference site(s).

Indices of Biological Integrity and HGM functional models require considerable time for development. Yet actual wetland assessment with these methods typically only requires

[1] http://www.epa.gov/wetlands/science/hgm.html.

one relatively short site visit (Bartoldus, 1999). For example, the Penn State sampling protocol for HGM functional assessment (Wardrop et al., 2004) requires one site visit and may also be used as a rapid assessment method. In our review of wetland assessment methods (Haering and Galbraith, 2010) we found only two Level 3 evaluation methods that required more than one site visit: the reference wetlands for the Hydrogeomorphic Approach to Functional Assessment for Piedmont Slope Wetlands (Vasilas, 2006) and the current Virginia Institute of Marine Science (VIMS) method (personal communication, Kirk Havens).

For the initial phase of the MDE Level 3 template development study, we reviewed studies in which wetland hydrology, biogeochemistry/soils, and/or habitat were examined by more intensive methods that required more than one site visit. We focused on relatively recent studies that were applicable to the Mid-Atlantic region, preferably those in which at least one year of data was collected.

Wetland Classification

Several wetland classification systems are used in Maryland:

- **Maryland Coastal Wetland system**: Oldest system currently used in Maryland. Classifies tidal wetlands only by salinity, range of tidal inundation and plant community (MDE Wetlands and Waterways Program, 2008).

- **U.S. Fish and Wildlife Service (USFWS) system** (Cowardin et al., 1979): Classifies both wetlands and deepwater habitats by ecological system: marine, estuarine, riverine, lacustrine, or palustrine. Systems are further divided into ecological subsystems, and then into classes based on either vegetation or substrate type (for areas with <30% vegetative cover). This system is used to classify wetlands via aerial photos for National Wetlands Inventory maps, but does not include many wetland characteristics that are important in functional evaluation (Brinson, 1993), particularly for non-tidal wetlands.

- **Key Wildlife Habitat system** (Maryland Department of Natural Resources, 2005). Used to identify habitats used by species of Greatest Conservation Need. Ten wetland wildlife habitat types are identified under this system: floodplain forests, upland depressional swamps, Carolina (Delmarva) bays, vernal pools, forested seepage wetlands, bog and fen wetland complexes, nontidal shrub wetlands, tidal shrub wetlands, nontidal emergent wetlands, and tidal marshes.

The wetland classification system used for this template is **Maryland's Draft Wetland Classification**, which has been outlined by the Maryland Department of the Environment (MDE) Wetlands and Waterways Program (2008), and is summarized in Table 1. The draft classification is modified from the HGM classification system of Brinson (1993), with some subclasses including more than one HGM class based on landscape position. For example, the Riparian Headwater class includes slope and depression wetlands that are associated with the floodplain. Maryland's draft classification also includes hydroperiod, which is not a component of the HGM system. The classification system may be cross-referenced with the wetland portion of the Key Wildlife Habitat system used by the Maryland Department of Natural Resources (2005).

Table 1. Draft regional subclasses for Maryland's wetlands (adapted from MDE Wetlands and Waterways Program, 2008).

Maryland Wetland Class	HGM Class	Brief Description	Hydrology: 1) source 2) hydrodynamics 3) hydroperiod	Key Wildlife Habitat
Tidal Freshwater	Estuarine fringe	0 – 0.5 ppt salinity	1) Overbank flow from channel 2)Bidirectional, horizontal, vertical 3)Diurnal	Tidal Shrub Wetlands Tidal Marshes Floodplain Forest
Tidal Estuarine	Estuarine fringe	> 0.5 ppt salinity	1) Overbank flow from channel 2)Bidirectional, horizontal, vertical 3)Diurnal	
Nontidal Riparian Headwater Complex	Riverine, Slope, Depressions	Riparian zone of waterway, floodplain, and transitional upland fringe. ≤ 3rd order mosaic of low/high gradient streams, depressions, toe-slopes	1) Overbank, groundwater, surface runoff 2) Bidirectional, horizontal, vertical 3) Variable	Floodplain Forest Nontidal Shrub Wetland Nontidal Emergent Wetland Forested Seepage Wetland Bogs and Fens Vernal Pools
Non-tidal Riparian Mainstem Complex	Riverine	Riparian zone of waterway, floodplain, and transitional upland fringe. > 3rd order mosaic of low/high gradient streams, depressions, toe-slopes	1) Overbank, groundwater, surface runoff 2) Bidirectional, horizontal, vertical 3) Variable	Floodplain Forest Nontidal Shrub Wetland Nontidal Emergent Wetland Vernal Pools
Seasonal Flat (mineral soil) • **Connected** • **Isolated**	Mineral Flats	Broad, flat areas with poor drainage	1) Precipitation, groundwater, overbank 2) Vertical 3) Temporarily to semi-permanently flooded	
Peatland • **Connected** • **Isolated**	Organic Flats, Depressions	Broad, flat areas or depressions with sustained saturation and deep peat	1) Precipitation, groundwater 2) Vertical 3) Saturated, semi-permanently flooded	Bogs and Fens
Isolated Depressional	Depressions	Topographic low area lacking hydrologic connection to riparian tidal waters	1) Precipitation, Groundwater, surface run-off 2) Vertical 3) Temporarily, seasonally, to semipermanently flooded	Upland Depressional Swamps Vernal Pools Carolina Bays
Isolated Seepage Slope	Slope	Discharge area lacking observable surface connection to riparian or tidal waters	1) Groundwater 2) Unidirectional, horizontal 3) Saturated most or all of the year	Nontidal Shrub Wetlands Nontidal Emergent Wetlands
Constructed or Incidental	Any class	May become any of above classes after wetland matures	Any of above sources	Can include many types.

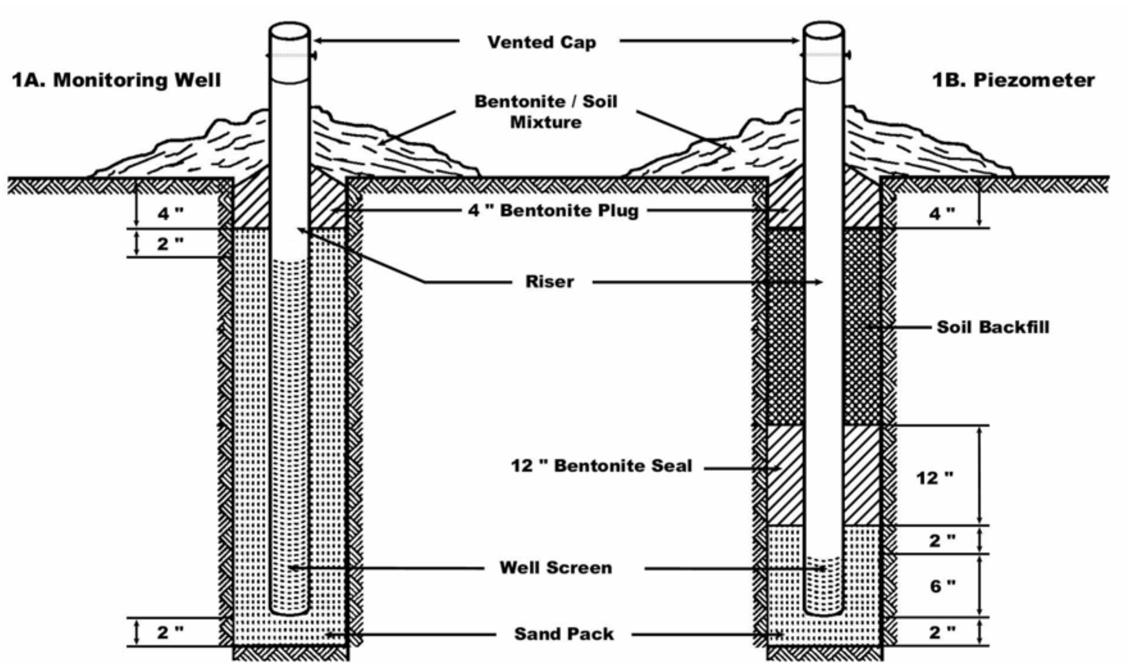
Hydrology

Wetland hydrology is usually considered to be the single most important factor affecting other wetland functions (Zedler, 2000). In most wetland assessment methods, however, hydrological conditions are approximated by using qualitative indicators such as observable drainage patterns, evidence of surface flooding and so forth, rather than quantitative measurements. These indicators often do not accurately reflect wetland hydrology (Ehrenfeld et al., 2003).

Quantifiable wetland hydrologic variables include, among others, water level, hydroperiod (frequency and duration of saturation), hydropattern (hydroperiod plus extent and timing of saturation), and hydrologic inputs and outputs (U.S. EPA, 2008a). Hydrologic inputs into a wetland may come from precipitation, overland flow, surface water inflow, groundwater discharge, and/or tidal inflow. Hydrologic outputs may include evapotranspiration, surface water outflow, groundwater recharge, and/or tidal outflow (Vasilas, 2004; U.S. EPA, 2008a).

Typically, water levels in wetlands are measured with wells or piezometers, although water levels in wetlands with permanent standing water can be measured with staff gauges. Water table observation wells are usually screened from just below the soil surface to the bottom of their installed depth so that water levels within the well supposedly reflect the actual "water table" in the surrounding unconfined soil aquifer system. Piezometers are open screened at a set depth increment (usually at the bottom) and sealed above that point, so water levels in piezometers reflect water pressure/potential at the open screened increment (Figure 1).

Figure 1. Diagram of installed monitoring well and piezometer. From Sprecher (2000).

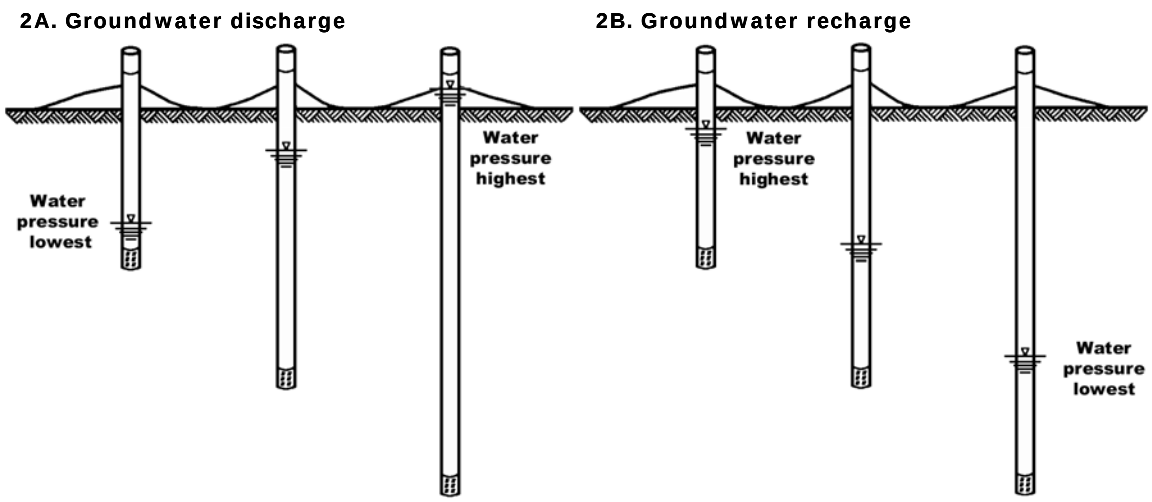


Water table observation wells should installed so that their screened interval is clearly above impermeable or slowly permeable soil layers that may cause perched water tables (epiaquic conditions), while piezometers can be installed above, within and below these layers to properly detect perching or epiaquic conditions. Piezometers are usually installed in nested sets at various depths in order to measure groundwater flow direction and rate, and differential water potentials in different soil layers (Sprecher, 2000). The water levels within a piezometer will only measure the actual surrounding water table levels if there is no vertical groundwater gradient or if the open screened section of the piezometer intersects the top of the local water table (Faulkner et al., 1989).

As shown in Figure 2, piezometer nests are particularly valuable for determining whether or not significant local groundwater vertical gradients exist at given location. Where the apparent water level (potentiometric level) in a piezometer nest increases with depth of the open screened increment (Fig. 2a), this is indicative of net groundwater discharge (or upwelling) at a given location. This situation can represent a significant source of groundwater ***inputs*** to given wetland or monitoring location. Frequently, wetlands in lower toeslope or riparian positions of larger watersheds receive significant groundwater inputs or discharge. Conversely, where the apparent water levels fall with open increment depth for a given set of piezometers, this is evidence of groundwater recharge and represents a significant pathway for potential ***losses*** of groundwater downward. Wetlands that occur on broad upland summits or along certain sloping positions are commonly characterized by being significant contributors to groundwater recharge.

Water table wells and piezometers can also be used to estimate lateral groundwater gradients and flow regimes when three or more are installed within a wetland area of interest assuming they are installed with appropriate spatial coverage. With five or more locations, certain inferences of the actual topography of the local water table surface can also be made along with greater insight derived into local lateral gradients. Thus, combinations of water table observation wells and piezometer nests within a given site allow the trained observer to interpret both the local lateral gradient (e.g. the slope of water table or potentiometric surface) and whether or not significant discharge or recharge is occurring. It is also important to note that significant differences in the direction and magnitude of vertical gradients can be found within a given wetland. In practical terms, this means that certain portions of a given wetland may be receiving groundwater discharge while other portions may be recharging groundwater.

Figure 2. Water levels in piezometers reflecting a groundwater discharge area and a groundwater recharge area [adapted from Sprecher (2000).] Groundwater discharge (2A) is indicated by increasing potentiometric water level in deeper piezometers, while groundwater recharge (2B) is indicated by decreasing potentiometric water level in deeper piezometers.



For this report, we examined a number of studies in which wetland hydrology was quantified during repeated site visits or data collection periods (Table 2). Some were complex hydrological analyses done on one site using a number of wells and piezometers (Harvey et al., 1987; Moorhead, 2001), while others used one water level well per site to collect data on timing and duration of saturation for many sites.

Table 2. Selected wetland hydrology studies.

Reference	Location and wetland type	# Sites	Study length	Goal of study	Methods used
Brooks and Hayashi, 2002	Vernal pools (isolated depressions) in MA	34	3 growing seasons for 24 pools; 1 growing season for 10 pools	Determine relationship of depth - area - volume and hydroperiod	Water depth in pools was measured on 1 m grids across surface while pools were at maximum area and depth. Data was used to estimate the relative basin surface elevation and a digital elevation model was generated using the Surfer program. This was used to calculate pool area and water volume at several depths. Presence or absence or surface water was recorded during semi-regular visits during the growing season and used to approximate hydroperiod.
Cahoon et al., 1995	Coastal and estuarine tidal wetlands in MS, NC, and FL	4	2 years	Compare marsh accretion and subsidence to the rate of relative sea-level rise.	Vertical accretion was measured with feldspar marker horizons. Surface elevation change was measured with a sedimentation erosion table (a portable mechanical leveling device for measuring the relative elevation change of wetland sediments”). Rates of subsidence of the upper 3-5 m of marsh were calculated as the difference between vertical accretion and surface elevation change. Local relative sea-level rise data was taken from the literature.
Cole et al., 1997	Riparian depressional (8), headwater floodplain (4), mainstem floodplain (5), slope (7) wetlands in the Ridge and Valley province of PA	24	3 growing seasons	Characterize wetland hydrologic behavior by HGM class by determining median water table depth, range of water table fluctuations, and time water was in the root zone (30 cm).	1-5 paired sets of wells and piezometers were installed in each wetland. Locations of sets were determined by direction/length of perceived hydrologic gradient. Wells were installed to a max of 1.5 meters. If restrictive (clay) layers were present, wells were installed above layer and piezometers below. Piezometer data used only to determine if ground water was a potential source of water for the wetland. Water levels measured monthly during the growing season for 3 years. Precipitation data was obtained via weather records.

Table 2, continued. Selected wetland hydrology studies.

Reference	**Location and wetland type**	**# Sites**	**Study length**	**Goal of study**	**Methods used**
Cole and Brooks, 2000a (follow-up to Cole et al., 1997)	Riparian depression (11), slope (9), headwater floodplain (6), and mainstem floodplain (4) wetlands in the Ridge and Valley Province of PA	30	Approx 1.75 to 4.75 years	Characterize wetland hydrologic behavior by HGM class by determining median water table depth, range of water table fluctuations, and time water was in the root zone.	Groundwater levels were determined with shallow groundwater wells ≤ 1.5 m which automatically recorded water levels every 3-6 hours. Smaller wetlands had 1 well, while larger wetlands had 2 placed along the hydrologic gradient. Precipitation data was obtained via weather records.
Cole and Brooks, 2000b	Natural (2) and created (2) mainstem floodplain wetlands in Central PA	4	Approx. 2 years	Compare hydrologic behavior of natural vs. created wetlands by evaluating median depth to the water table, range of water table fluctuation, percent of time water was within the root zone (30 cm).	At each site, 1 water level recording well was installed at river edge and 1 on a transect perpendicular to river. Wells were installed to 25-77 cm (depending on site soil characteristics). Water level recorded every 6 hours.
Ehrenfeld et al., 2003	Riverine, flat, depression, slope, and flat-riverine forested wetlands in urban/suburban areas of northeastern NJ	10	2.5 years	Compare quantitative hydrology to qualitative hydrology indicators Quantitative measurements included median, maximum, minimum, and coefficient of variation of water levels, period of time water was in the rooting zone (30 cm), estimation of "flashiness", and others.	Sites had three sets of paired wells and piezometers each, installed along perceived hydrologic gradient to a depth of 70 cm. Wells were read manually every 2 weeks. Groundwater discharge (piezometer levels higher than the wells) and recharge (piezometer levels lower than the wells) patterns were assessed by comparing the water levels in each piezometer with the adjacent well. Precipitation data was obtained from weather records.

Table 2, continued. Selected wetland hydrology studies.

Reference	Location and wetland type	# Sites	Study length	Goal of study	Methods used
Fennessy et al., 2004	Natural (9) and constructed (10) mixed emergent wetlands in Ohio	19	2 growing seasons (April-May to end of September)	Determine mean, median, maximum, minimum ground water levels, % time water was found within root zone (upper 30 cm of soil), and a "flashiness" index.	Water levels were determined with shallow groundwater wells with surveyed elevation which automatically recorded water levels every 12 hours. Typically, 1 well was installed per site. The depth of well was determined by the distance to impermeable clay layer.
Gamble et al., 2007	Riverine and depressional wetlands in Central Ohio	22	1 year	Determine flood storage capacity of urban wetlands	Wetland morphometry was determined via level and GIS survey. Perimeter was determined via measuring the boundaries of jurisdictional delineation. Water levels were determined with shallow groundwater wells with surveyed elevation which automatically recorded water levels every 12 hours. 1 well was installed per site except in 3 sites which were composed of 2 hydrologically distinct areas. Well depth was determined by the distance to impermeable clay layer. A 3-D model of wetland basin morphometry was used to calculate area and water volume at several depths (via Surfer 8.05 program). Storage volumes were compared to both precipitation data and flow data for typical streams.
Harvey et al., 1987	Tidal estuarine wetland in VA	1	4 tidal cycles over 2 months	Assess subsurface hydrology and pore water dynamics in the creekbank zone of a tidal wetland.	1 well and 3 nested piezometers (installed at 25, 45 and 75 cm depth) in 5 locations, plus 3 additional wells in a surveyed transect perpendicular to tidal creek. Water levels in both piezometers and wells were measured manually during 4 complete tidal cycles.

Table 2, continued. Selected wetland hydrology studies.

Reference	Location and wetland type	# Sites	Study length	Goal of study	Methods used
Korfel et al., 2009	Natural (6) and created vernal (10) pools (isolated depressions) in Ohio	16	1 growing season	Compare pool volume, period of inundation, and hydrologic connectivity between groundwater and surface water in natural and created vernal pools.	2 water level recorder wells were installed in 6 pools, 1 in the deepest part of pool and 1 just outside the pool surface, and levels were recorded hourly. Water levels in remaining pools were measured with staff gauges in deepest part that were read weekly. Wells were installed approximately 50–80 cm deep depending on the soil profile (wells were not installed below an impermeable clay layer). Precipitation data was acquired via weather records. Perimeter and surface area of each pool were determined by GPS. Pool area was used to calculate pool volume throughout the season for depressional wetlands: volume (m3) = area (m2) × max depth (m) × 0.3135.
Mack and Micacchion, 2006	Mitigation banks in lake plains of northwest Ohio, till plains of central Ohio, and glaciated Allegheny plateau of northeast Ohio	33	≥ 1 year	Determine whether mitigation banks had established wetland hydrology	Surface water depth was measured during the summer with a measuring tape. Groundwater levels were determined with shallow groundwater wells with surveyed elevation which automatically recorded water levels every 12 hours. Well depth was determined by the distance to impermeable soil layer. Wells were located just upslope of the area of maximum inundation at sites with substantial inundation and in representative locations for areas with saturated soils.
Moorhead, 2001	Appalachian Highland fen and surrounding floodplain and slopes in NC	1	4.5 years	Evaluate the hydrologic linkage between a mountain fen, surrounding floodplain, and adjacent hillslopes by measuring water table levels and evaluating piezometer data.	Wells/piezometers installed: • 12 shallow wells in floodplain/fen complex. • 10 wells along 2 transects from adjacent slopes into fen. • 6 piezometers in the fen and 3 in the floodplain to evaluate groundwater discharge/recharge. Wells were installed to 84 cm; piezometers to 137 cm. Water levels of wells and piezometers were measured manually every 1 or 2 weeks. Precipitation was measured via rain gauge.

Biogeochemistry

Biogeochemical functions in wetlands include nutrient cycling as well as removal of nutrients, sediment, trace metals, carbon sequestration, and particulate and dissolved organic matter from surface water and groundwater. For the purposes of this review, we will describe procedures for estimation of nitrogen (N) and phosphorus (P) cycling and removal; sediment removal; and for determining hydric soil morphology and redox status.

Nutrient Cycling

Vasilas (2004) and Vasilas et al. (2008) have summarized nutrient removal and cycling in wetlands:

- Wetlands remove nutrients via uptake by plants, algae, and microorganisms, chemical precipitation, adsorption to soil, and denitrification (the reduction of nitrate to gaseous N forms.)
- Nutrient cycling processes in wetlands include:
  - Mineralization: change from organic to inorganic form.
  - Immobilization: change from inorganic form to organic form
  - Transformation: change from one inorganic compound to another inorganic compound.
- Nitrogen cycling in wetlands:
  - Nitrogen enters wetlands via surface and ground waters, precipitation, and N fixation [conversion of N_2 gas to ammonium (NH_4^+) by some species of bacteria and algae].
- Nitrogen removal in wetlands primarily takes place via denitrification, the reduction of nitrate to gaseous N forms. Denitrification is bacterially mediated and requires nitrate, organic carbon (C) and an anaerobic environment. Dissolved ammonium and organic N in water must undergo nitrification (oxidization to nitrate) before removal via denitrification. These two processes require a fluctuating water table or locally oxidized conditions. Dissolved organic N must undergo ammonification (oxidation to ammonium) and nitrification before removal via denitrification.
- Phosphorus cycling in wetlands:
  - Phosphorus enters wetlands when adsorbed to solids (sediment) or in dissolved form (organically complexed or as ortho-P).
  - Phosphorus is not lost via conversion to gas, but can be removed via precipitation with Fe and Al under acidic conditions and with Ca under alkaline conditions, by adsorption to soil, and by biological uptake and subsequent conversion to stable organic compounds.
- Wetlands, particularly those with a fluctuating water table, are typically quite efficient at removing N (70-90% average removal), but tend to be less efficient than uplands in removing P (40-50% average removal).

The U.S. EPA (2008b) has outlined recommended procedures for determining nutrient impact on wetlands. These procedures do not measure actual biogeochemical processes, but rather assess the source material and end products of these processes. Although some researchers have attempted to quantify biogeochemical processes in wetlands [for example, Hefting et al. (2004) and Jordan et al. (2007) quantified denitrification enzyme activity and nitrous oxide emissions, and Stander and Ehrenfeld (2009a) assayed the rates of net N mineralization, net nitrification, and denitrification], these techniques can be difficult and expensive, and tend to only provide information on a narrow set of processes that may not adequately reflect overall biogeochemical functions.

For example, the U.S. EPA (2008b) recommended procedures consist of:

1. Establishing background levels of nutrients. This is most commonly done by using an unimpacted or minimally impacted reference wetland, but if all wetlands in the watershed are impacted, nutrient levels at lower soil depths within a wetland can be used as background. Under these conditions, minimally impacted sites can be used as "reference" sites. Once the reference sites are established, spatial and temporal variability in selected indicators should be monitored to determine the ranges in values. This initial database is essential to establish nutrient criteria.

2. Sampling water and measuring water depth at inflow and outflow points and other locations within the wetland repeatedly in order to determine nutrient removal within the wetland and seasonal changes in water nutrient concentrations.

3. Sampling litter layers and surface soils at selected locations within the wetland to determine overall nutrient concentrations in the wetland. (Note that these levels can be quite variable depending on input location.) The nutrient levels in the litter layer tend to reflect the most recent inputs, while soil nutrient levels tend to reflect long-term nutrient accumulation. Soil samples must be taken by collecting soil cores so that bulk density can be determined and nutrient concentrations can be expressed on a volume rather than a mass basis, allowing comparison among soils with varying textures and organic matter levels.

4. Collect the following data (at a minimum):

- **Water:**
  - Water depth
  - Total N
  - Total P

- **Litter layer**:
  - Total C
  - Total N
  - Total P

- **Soil:**
    - Bulk density
    - Organic matter
    - Total C
    - Total N
    - Total P
    - Extractable ammonium N (2M KCl)
    - Extractable P (Mehlich I or III)
    - Oxalate-extractable Fe and Al
    - Extractable Ca, Mg, and K (Mehlich I or III)

Presumably, these samples will be taken at a sufficient number of locations within a given wetland to allow for statistical comparisons against either reference sites or against temporal samples taken from the same wetland (and sites) over time.

Sediment Accretion

The sediment accretion process in wetlands involves the accumulation of both organic and inorganic material at the surface via the process of sedimentation (U.S. EPA, 2008b). The sedimentation rate within a wetland will depend on factors such as sediment particle size and density, hydrologic residence time, vegetation density and uniformity, wind or wave strength, and water flow patterns. In wetlands without channelized flow, most sedimentation tends to occur nearest the sediment source (Fennessy et al., 1994).

Two methods are often recommended for approximating annual sedimentation rates in wetlands (Kleiss, 1993; Steiger et al., 2003):

1. Marker horizons created with feldspar clay or other material.
2. Flat sediment plates made from roughened plexiglass or tile.

In the marker horizon method, feldspar clay or another material is spread over the wetland surface in a thin layer in replicated and marked plots. Harter and Mitsch (2003) used 1 cm layers over 0.25 m^2 plots. Cahoon and Turner (1989) successfully used feldspar marker horizons to measure relatively recent (6 months to 1 year) sedimentation rates in Louisiana tidal wetlands by taking cores through the sediments over the marker horizon with thin aluminum soft drink cans with the top and bottom removed. Plexiglass tubes or other methods can also be used for coring (Cahoon et al., 1995; Steiger et al., 2003). Feldspar marker horizons were also used by Cahoon et al. (1995) to measure shallow subsidence in tidal marshes in Louisiana, Florida, and North Carolina; by Darke and Megonigal (2003) to measure vertical accretion in freshwater tidal marshes on the Mattaponi River of Virginia; and by Harter and Mitsch (2003) to measure sedimentation rates in constructed freshwater wetlands in Ohio.

The main advantage to using marker horizons (besides the fact that they require no specialized equipment) is that they can be resampled to determine semi-annual or annual sedimentation rates if care is taken to clearly mark and recall previous sampling areas (Cahoon and Turner, 1989; Kleiss, 1993). Disadvantages include possible resuspension of the marker horizon, disturbance by faunal pedoturbation, or mixing of the marker with sediments so that it becomes invisible (Cahoon and Turner, 1989). Harter and Mitsch (2003) tested different types of marker horizons in freshwater wetlands on a clayey substrate and found that the relocation rate of marker horizons made of silica sand was much higher than those made of feldspar, apparently because of the contrasting texture of the marker horizon and the wetland sediments.

The advantage to using sediment tiles or disks is that deposited sediments can be easily collected for chemical or physical analysis after sediment depth is measured. Sediment tiles were successfully used by Darke and Megonigal (2003), and Morse et al. (2004) to measure monthly sediment accumulation and sediment characteristics in freshwater tidal wetlands along the Mattaponi River in Virginia. Round anchored sanded plexiglass disks were used by Wardrop and Brooks (1998) to measure annual sedimentation and characterize sediments in 25 Pennsylvania wetlands. [Kleiss (1993) has a schematic diagram with specifications for constructing plexiglass sediment disk traps.] One problem with the use of sedimentation disks, however, is that precipitation may remove light coatings of sediment (Kleiss, 1993).

Sediment plates would likely be best used in places where quantification of sediment properties is important (for example, determining nutrient sources). Marker horizons would be more appropriate in areas where periodic quantification of sediment accretion rates is desired, such as tidal wetlands. Both methods could be used together, as in Darke and Megonigal (2003), if desired.

Determining sedimentation rates is particularly important in tidal wetlands. Tidal wetlands accumulate material at the surface via tidal or storm sedimentation, peat accumulation, and fluvial sediment supply and lose surface elevation via compaction, tidal sediment export, decomposition, and subsidence (Fitzgerald et al., 2009). Tidal wetlands can only persist over time if they accumulate material at the surface at a rate equivalent to compaction, export, decomposition and sea level rise. Recent rapid increases in sea level may mean that many of these wetlands will not be able to sustain surface levels, and will "drown" or be transformed into mud flats

According to Cahoon and Guntenspergen (2010), the time required for a tidal marsh to become subtidal can be estimated from rate of sea level rise and the elevation of the marsh relative to low and high tide by:

1. Determining *elevation capital*. Elevation capital is "the position of the wetland relative to the lowest elevation at which plants can survive," and can be approximated by determining tidal range and wetland surface elevation relative to sea level. Tidal marshes with substantial vertical tidal range have more elevation capital, as do marshes which are located at an elevation that is in the upper portion of the range at which marsh plants can survive. Marshes with higher elevation capital are more resistant to sea level rise.

2. Determining trends in elevation relative to sea level, or whether the rate of vertical accretion via sediment deposition and organic matter accumulation in a tidal marsh is keeping pace with sea level rise. If vertical accretion is not keeping pace with local sea level rise, this results in an *elevation deficit*. Quantification of an elevation deficit requires measuring changes in local sea level and in marsh surface elevation with time. Changes in marsh surface elevation can be determined with a piece of equipment called a Surface Elevation Table (Cahoon et al., 1995), which is a "portable mechanical leveling device for measuring the relative elevation change of wetland sediments."[2]

Hydric Soil Morphology and Redox Status

Wetland soils are responsible for many of the biogeochemistry functions within wetlands, particularly those which involve redox reactions. Detailed and accurate soil profile and morphological descriptions may be used to infer soil redox status and provide certain information about local hydroperiods on sites that have not been artificially drained or otherwise hydrologically altered. Furthermore, this detailed soil morphological information and data can then be utilized for determination of Hydric Soil Indicators (HSI) as described below which in most instances, can be taken as "proof positive" of hydric soil conditions in the field.

Standard methods for describing hydric soils are well established. Detailed instructions for standard soil description and sampling procedures are given in Schoeneberger et al. (2002). Specific instructions for describing hydric soils, including hydric soil indicators, may be found in USDA-NRCS (2010).

In some cases, the presence or absence of hydric soil indicators may not be a reliable way to determine if the soils in constructed wetlands are indeed hydric. Although redox features develop fairly quickly in created wetlands in Virginia mitigation sites (Daniels and Whittecar, 2004), the soils in created wetlands may either (1) contain either no fully developed hydric soils indicators or (2) possess relict hydric soil features from before mitigation (MDE Wetland and Waterways Program, 1997). Ideally, hydric soil development in constructed wetlands should be quantified by describing soils at the site before disturbance and immediately after final grading and then monitoring changes in soil morphology over time (Daniels and Whittecar, 2004). If and where this is not possible, IRIS tubes (Rabenhorst, 2008), can be used to quantify reducing conditions.

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[2] http://www.pwrc.usgs.gov/set/ Further details on the Surface Elevation Table are available at this website.

Habitat

Vegetation

A review of Level 3 vegetation sampling methods used in other Region III states (Table 3) showed that most methods used intensive plot or grid methods within the wetland to evaluate species richness and abundance. All these methods, however, required only one site visit. A minimum of two sampling visits, one in late spring and one in late summer, are suggested so that the seasonal variability of vegetation may be assessed (Mueller-Dombois and Ellenberg, 1976; DeBerry and Perry, 2004).

Table 3. Selected Level 3 wetland vegetation assessment procedures for other EPA Region III states.

State	Reference	Method ID	Method summary
DE	Jacobs et al., 2008	Delaware Comprehensive Assessment Procedure (modified HGM)	Data is collected on one or more small round plots in each zone of dominant vegetation in the assessment. Number of plots depends on percent wetland that is covered by dominant vegetation. Number, species, and diameter of trees; number, species, # stems, and diameter of saplings, number, species and # stems of shrubs, number and species of vines, presence or absence of *Rubus* (blackberry), and % cover and species of all understory species.
PA	Miller et al., 2004 (IBI); Miller and Wardrop, 2006 (FQAI); Wardrop et al., 2004 (HGM)	Plant-Based Index of Biological Integrity; Floristic Quality Assessment Index; and HGM standard procedure	Data is collected on nested quadrats laid out along an evenly spaced grid. Herbaceous species % cover estimated within a 0.5 m^2 quadrat; herbaceous species richness, shrub species richness and shrub volume within a 3 m radius plot; tree species richness and dbh within an 11.6 m radius plot.
VA	Havens et al., 2001	Hardwood flats HGM model	Data is taken on 10 m radius plot. Number and species of herbaceous plants, shrubs, saplings, and trees is recorded and exotic species are noted.
VA	Personal communication, Kirk Havens	VIMS Level 3 method for non-tidal wetlands	For wetlands that have adjacent stream flow only: species and dbh of all woody stems >1m tall and < 5 cm DBH is recorded in three 1.9 m radius plots.
WV	Kordek, 2008	West Virginia Level 3 method	Data taken on small (~10 m x 10 m) releves (plots) in representative areas of the wetland using an adapted version of the method of Vanderhorst et al. (2008). Height and % cover is assessed for emergent trees, canopy trees, sub-canopy trees, tall shrubs, short shrubs, herbaceous vegetation, non-vascular vegetation, floating vegetation, and submerged vegetation. Individual species are characterized by number (if appropriate), stratum, dbh (if appropriate), and % cover. Presence of invasive species is recorded.

We propose a detailed vegetation survey that involves two sampling dates (late spring and late summer), and that will produce a quantitative description and statistical comparison of the vegetation assemblages within and between sites.

The survey will produce the following:

1. Vegetation composition data (dominant species, species richness and evenness, species diversity, presence of rare or endangered species),
2. Comparison of vegetation assemblages of a site to that of a reference site,
3. Measurement of alpha (species), beta (within sites), and gamma diversity (between sites) for Maryland wetlands.
4. The potential for time series analysis of succession in created and restored wetlands.

Wildlife

Many currently used wetland assessment methods evaluate wildlife habitat potential based on the structural features of wetland vegetation and/or surrounding land use rather than attempting to quantify actual use by wildlife (Haering and Galbraith, 2010). An exception to this is wetland bioassessment methods such as indices of biological integrity (IBI's). Although IBI's are condition measurements, the methods used to develop IBI's can also be used to quantify wildlife use of wetlands for functional assessment.

Macroinvertebrates are probably the most common biological assemblage used to develop IBI's, but although they are fairly easy to sample, they can be very difficult to identify to the genus level (U.S. EPA, 2002a; Laubscher and Conklin, 2004; Hatfield et al., 2006). Two other species groups often used in wetland biological assessments are birds and amphibians (U.S. EPA, 2002a; U.S. EPA, 2003).

Birds

Birds are commonly used in direct assessments of wildlife habitat in wetlands because bird species richness and abundance is often an indicator of habitat fragmentation, changes in land use, and change in habitat structure in the landscape surrounding the wetland (U.S. EPA, 2002b; DeLuca et al., 2004). Bird populations are also comparatively easy to examine as compared to other wildlife species groups. No physical sampling is required and identification is relatively simple.

The U.S. EPA (2002b) recommends that bird populations in wetlands be quantified using either point counts or area counts. Point counts can be used in all wetlands. Bird species that are seen and heard from fixed points at a given distance apart within a wetland are tallied for a limited amount of time. Area counts are appropriate only for open herbaceous wetlands such as salt marshes or open water area of other wetlands, and are used to survey waterbirds rather than songbirds. Identification is also from a fixed point, but as many points as necessary to cover the entire wetland may be used as long as they do not overlap. In our survey of regional studies, we found that point counts were by far the most commonly used method, and that they were used in both tidal (Table 4a) and non-tidal (Table 4b) wetlands.

Standard protocol for performing point counts includes the following (U.S. EPA, 2002b):

- Birds that are seen or heard within a given radius are counted for 5 or 10 minutes at each point. (In some point count methods, the distance to each bird is also estimated.) The studies we surveyed used a 50 m radius point count.
- To compare data from separate wetlands, the same number of points and same number of visits must be made to each site. Wetland should preferably be visited on the same day or on consecutive days at the same time of day. If two or more sites are visited on the same day, the order of site visits should be changed during the next visit.
- Counts of secretive bird species can be augmented by callback surveys. These involve playing recorded bird calls and documenting bird responses.
- Guidelines for timing of site visits are:
    - Counts should be made at least twice per season, preferably during the breeding season (approximately May-July).
    - Sites should be visited during the four hours after sunrise, unless nocturnal species are the focus of the count.

More guidelines for point counts are given in Ralph et al., (1995).

Table 4a. Avian point count methods from Mid-Atlantic tidal wetland studies.

Reference	**Number, type, location of wetlands studied**	**Point count methods**
DeLuca et al., 2004	96 tidal estuarine wetlands in 30 Chesapeake Bay watersheds	50 m radius, 5 minute, double observer point counts were used. 1 point count location was used to sample wetlands <2 ha, 2 for wetlands between 2 and 7 ha, and 3 for wetlands >7 ha. Point count locations were located 50 m from the upland edge of the marsh and 150 m from each other in representative portions of the marsh. After the point count a bird callback survey was conducted from a centrally located point count location (unlimited radius). Bird* calls were broadcast for 1minute per species with 30 seconds listening time between species calls and one minute of listening time at the end. Two visits per site (at least 2 weeks between visits) were made during breeding season (15 May to 18 July) between 6 AM and 11 AM. * Black rail (*Laterallus jamaicensis*), least bittern (*Ixobrychus exilis*), common moorhen (*Gallinula chloropus*), Virginia rail (*Rallus limicola*), clapper rail (*Rallus longirostris*), sora (*Porzana carolina*), American bittern (*Botaurus lentiginosus*), and pied-billed grebe (*Podilymbus podiceps*).
McLaughlin, 2007	8 tidal estuarine wetlands on the eastern (4) and western (4) sites near the mouth of the Nanticoke River in MD.	Point count protocol was adapted from DeLuca et al. (2004), and was similar except that 1 point count and 1 site visit was made per wetland assessment area.
Desrochers et al., 2008	11 sets of paired created and natural (reference) salt marshes (22 total) on the SE VA Coastal Plain	50-m radius 10 minute point counts were used. Sampling circles (or semi-circles) were placed to allow the maximum number per wetland (1 to 3); but the same number of counts and shape of sampling circles were used in each set of paired natural marshes and created marshes.) Three visits per site (at least 10 days between visits) were made during breeding season (15 May to 15 July) between 6 AM and 9:20 AM.

Table4b. Avian point count methods from Mid-Atlantic non-tidal wetland studies.

Reference	Number, type, location of wetlands studied	Point count methods
Snell-Rood and Cristol (2003)	11 created (6) and natural (5) bottomland hardwood wetlands in SE VA	50 m radius 10 minute single observer point counts were used. Two point count locations were used per site with random placement at least 100 m from one another and more than 50 m from site edge. Two visits to each site were made during breeding season (28 May to 6 July): one at dawn (4:30–5:30 AM) and the other no later than 08:30. Each site was visited once between 28 May–15 June and once between 16 June–6 July) to allow for seasonal variations.
Balcombe et al, 2005[3]	15 mitigation (11) and reference wetlands (4) in the Ridge and Valley, Central Appalachians and Allegheny Plateau ecoregions of WV	50 m radius 10 minute single observer point counts were used. One to five point count locations were used per site, with locations being ≥250 m apart. Two visits to each site (at least 10 days between visits) were made during breeding season (5 May to 27 June) between 30 minutes before sunrise and 10 AM. After the point counts were completed, a bird callback survey was conducted from each point count station. Bird* calls were broadcast for 50 seconds per species with 40 seconds listening time between species. *Virginia rails (*Rallus limicola*), king rails (*Ralluselegans*), soras (*Porzana carolina*), American bitterns (*Botaurus lentiginosus*), least bitterns (*Ixobrychus exilis*), and pied-billed grebes (*Podilymbus podiceps*).
Veselka et al., 2010	151 emergent, scrub-shrub, and forested wetlands in the Ridge and Valley, Central Appalachians and Allegheny Plateau ecoregions of WV	Point counts were made using an adapted version of the methodology of Balcombe et al., 2005. One point count location was used per site, and although two visits to each site were made during breeding season (15 May to 1 July), only the highest count for each site was used in an attempt to increase species detection rate.
VIMS Level 3 method (personal communication, Kirk Havens)	Non-tidal wetlands in VA Coastal Plain	A stratified point counts method was used, in which the data collected at each point included site, date, start time, species of birds detected, distance from point center (within 50m, and >50m) of each detection, time period of detection (0-3, 3-5, 5-7, 7-10, and 10-15min), and detection method (visual, aural, both) Three visits per site were made during breeding season (late May to early July) from 0.5 and 4.5 hours after sunrise.

[3] A similar method is used in the West Virginia Level III method (Kordek, 2008).

Amphibians

Many amphibians require wetlands or open water to complete their life cycle. Larvae are aquatic but adults inhabit upland areas. Because of these habitat requirements, amphibians are very vulnerable to environmental changes and are often used as indicators of environmental disturbance (U.S. EPA, 2002).

According to Micacchion and Gara (2008), wetlands that provide amphibian habitat generally are freshwater depressional wetlands that are inundated during the amphibian breeding season and in which predatory fish are absent. Typically these wetlands have scrub-shrub or tree vegetation component. In Maryland, many amphibians require seasonally inundated isolated depressions such as vernal pools and Delmarva Bays for breeding (Maryland Department of Natural Resources, 2005), and direct assessment of amphibians by field sampling should be concentrated in these areas and timed accordingly.

There are several commonly used field sampling methods for amphibians. A combination of one or more of these methods may be necessary to sample amphibians at different stages in their life cycle.

- Funnel trapping. This method is most recommended by U.S. EPA (2002). It has been used extensively by the Ohio EPA in the quantitative portion of their amphibian surveys (Micacchion, 2004; Mack and Micacchion, 2006; Micacchion and Gara, 2008).
- Dipnet sweeps. These are more effective for anurans (U.S. EPA, 2002) This method has been used by Korfel et al. (2009), in the VIMS level 3 assessment method (personal communication, Kirk Havens), and is used the qualitative portion of the Ohio EPA method (Micacchion, 2004; Mack and Micacchion, 2006; Micacchion and Mara; 2008)
- Seining. This method was used to assess amphibian larva in a study on Delmarva Bays in Maryland (U.S. EPA, 2003; also on-line at http://www.epa.gov/wetlands/bawwg/case/md.html)
- Calling surveys. This method is for anurans (frogs and toads) only. It was used by Balcombe et al., 2005; in the West Virginia Level III method (Kordek, 2008), and in the VIMS Level III (personal communication, Kirk Havens).
- Pitfalls with or without drift fences. This method was used to assess amphibian adults in the Delmarva Bay study (U.S. EPA, 2003).
- Artificial cover such as cover boards. These work best for surveys of caudates such as salamanders and newts (U.S. EPA, 2002).

Repeated site visits, preferably over several years, are desirable when quantifying amphibian species richness and abundance since their numbers can fluctuate greatly from month to month depending on life cycle stage, and tend also to fluctuate from year to year depending on the percentage of the population that is breeding (U.S. EPA, 2002). Sampling visits should be planned around life cycle of the amphibians of interest, and wetlands should be sampled at least twice during the breeding season.

The Ohio EPA has developed a detailed collection protocol for amphibians in wetlands with seasonal inundation. It can also be used to sample macroinvertebrates, if desired. The method is fully described in Micacchion (2004), and involves quantitative sampling with funnel traps along with qualitative dipnet sampling. Wetlands are sampled three times every six weeks between late winter/early spring and late spring/early summer (exact dates depend on regional breeding cycles). The late winter/early spring sample is taken at the beginning of the amphibian breeding cycle, and is used to monitor breeding salamander and early breeding frog populations. A middle spring sample evaluates later breeding frogs and amphibian larvae from early breeders. A late spring/early summer sample is used to evaluate amphibian larvae.

Level 3 Method Calibration

As stated earlier, Level 3 methods are designed as relatively intensive assessments of either function or condition. Level 3 *condition* assessment methods such as Indices of Biological Integrity (IBI's) and Floristic Quality Assessment Indices (FQAI's) are commonly calibrated and scored by comparing them to independent measures of anthropogenic disturbance. For example, both the Pennsylvania Floristic Quality Assessment Index (Miller and Wardrop, 2006) and the Ohio Vegetation IBI (Mack, 2007; Mack, 2009) were tested by comparing them to, respectively, the Penn State Landscape/Rapid Assessment Method (Brooks et al., 2004) and the Ohio Rapid Assessment Method[4] (Mack, 2001). Condition categories were then adjusted as necessary to conform to the rapid assessments of condition.

The most frequently used Level 3 *functional* assessment methods are generally based on the HGM approach[5]. Although the indicators used in HGM assessment are often based on one site visit, HGM method calibration techniques can also be applied to more intensive assessments over extended periods of time.

HGM assessment methods produce mechanistic models in which "environmental characteristics found in a wetland are treated as variables in an equation" (Hruby et al., 1999). Data collected for each indicator is standardized by converting it to the same scale (typically 0-1) and then combined mathematically to produce an overall score for each function (see Tables 5 and 6 for an example of this). In the HGM method, each function is scored separately and the scores are not combined, so the assessment does not provide a single overall score for each wetland. Furthermore, scores from different regional HGM subclasses are not comparable. For example, scores for nutrient transformation within a depression cannot be compared to scores for nutrient transformation for riverine wetlands.

Hruby et al. (1999) and Adamus (2001) have outlined the steps required for developing a regional HGM assessment model.

1. Identify and define regional HGM wetland classes and subclasses.
2. Identify and define the functions performed by each wetland class.
3. Identify and define indicators and methods and construct assessment models that evaluate these functions.
4. Identify *reference sites*. Reference sites include all the sites of a regional subclass that will be assessed to calibrate the assessment model, and can include disturbed sites. Thirty to 50 sites are often used per HGM subclass (Adamus, 2001).
5. Collect data from reference sites and identify *reference standard sites*. In the classic HGM method, reference standard sites are always the *least altered* site

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[4] Although ORAM is not technically a measure of condition, scores from the portion of ORAM have been correlated with total ORAM scores.

[5] HGM methods such as the Delaware Comprehensive Assessment Procedure (Jacobs et al., 2008) assess condition rather than function.

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(Smith et al., 1995; Brinson and Rheinhardt, 1996). In the HGM method of Hruby et al. (1999), reference standard sites are those "*perform at the highest level for individual functions, regardless of level of alteration*." The HGM method of Adamus and Field (2001) requires that the user choose from either of the above types of reference standard sites.

6. Calibrate assessment models using reference site data. Scoring for the individual function for each site is accomplished by dividing the score for a site by the score for the reference standard wetland.
7. Verify and validate the assessment models.

Examples of indicators and scoring scales

Example 1: HGM method for assessing wetland functions in riverine and depressional wetlands in the lowlands of Western Washington (Hruby et al., 1999).

For HGM functional assessment of riverine and depressional wetlands in the lowlands of Western Washington (Hruby et al., 1999), the following functions were chosen for assessment (some functions are described as "potential" because actual measurements were not made):

- Potential for removing sediment
- Potential for removing nutrients
- Potential for removing heavy metals and toxic organics
- Potential for reducing peak flows
- Potential for decreasing downstream erosion
- Potential for recharging groundwater
- General habitat suitability
- Habitat suitability for invertebrates
- Habitat suitability for amphibians
- Habitat suitability for anadromous fish
- Habitat suitability for resident fish
- Habitat suitability for wetland-associated birds
- Habitat suitability for wetland-associated mammals
- Native plant richness
- Potential for primary production and organic export

The functions performed by each regional HGM subclass and indicators for each function were then identified by an assessment team. Indicators were limited to those that could be determined on a one-day site visit at any time of year. Functions and indicators were then refined using results from field data collection. The HGM models were then calibrated by using data from reference standard wetlands. As stated earlier, Washington State chose to use data from the wetland which provided the highest performance level for each function as the reference standard for that function, rather than choosing undisturbed wetlands as the reference standards. This was based on the assumption that "the highest level of performance of wetland functions will occur when a specific set of optimal environmental conditions are met, regardless of whether or not the wetland has been subject to human disturbance" (Hruby et al., 1999). This assumption was tested by assessing both "least altered" and "highest performing" reference standard wetlands. Results indicated that the least altered wetlands did not necessarily perform at the highest level for each function.

An example of the indicators and scoring scales used for the function "Removing Nutrients" in the Western Washington HGM method is shown in Table 5. The scoring scales for each variable used in the riverine and depressional HGM model for Washington State were developed by collecting field data on environmental characteristics on 86 reference sites (35 depressional outflow, 19 depressional closed, 12 riverine impounding, and 20 riverine flow-through). The data was tabulated, and 0 (lowest) to 1 (highest) scales were developed by using minimum and maximum data for each variable.

For example, the variable "*Veffectarea2*" in Table 5 below represents the "areal extent of the assessment unit (as a % of total) that undergoes changes between oxic and anoxic conditions" (Hruby et al, 1999). This was chosen because wetlands with a fluctuating water table provide the most optimum conditions for N transformation. The indicator for this was "the annually inundated area minus the area of permanent exposed inundation" (Hruby et al., 1999). The highest performing wetlands for this function were judged to be those which underwent complete seasonal inundation every year, but had no permanent areas of standing water. They were assigned a score of 1. Scores for other wetlands were based on the percent of the site area that was seasonally inundated without being permanently inundated. Because the indicators used had to be assessed relatively rapidly, scoring was based on observations only.

The variable *Vsorp* (Table 5) was based on the adsorption capacity of wetland surface soils. This was chosen because phosphorus is removed by adsorption, which is greater in soils with high clay or organic matter levels. This variable was assessed qualitatively so the scoring scale was simplified: wetlands with less than 50% non-clay mineral surface soils were rated 1; wetlands with 50-95% non clay mineral surface soils were rated 0.5; and wetlands with ≥95% non-clay mineral surface soils were rated 0. Further information on all the variables used in this method is detailed in Hruby et al. (1999).

Because the indicators used in this method are based on fairly rapid qualitative assessment, the scores for each indicator do not reflect the actual rate at which a process is being performed. In other words, a wetland which is rated 0.5 for adsorption capacity should not be assumed to have half the adsorption capacity of a wetland which is rated 1. The 0.5 score means that the wetland is performing the adsorption process at a "moderate", rather than "high" level.

To produce a total score or *index* for each function, scores for each variable were added, then multiplied by a factor (e.g. 2.70 for the "Removing Nutrients" function in Table 5), so that the total score for each function assessed equals 10. This value was then divided by the total score (index) from the reference wetland which had the highest score for that function.

Table 5. Example of indicators (variables) and scoring scales for the function "Removing Nutrients" in riverine impounding wetlands in the lowlands of Western Washington (adapted and simplified from Hruby et al., 1999).

Variable	**Description of variable**	**Description of Scaling**	**Score for Variable**	**Result**
Sssed	Assessment unit's index from the function "Removing Sediments"	Scaled Score:	Index for Removing Sediment	Index for Removing Sediment /10
Vsorp	The sorptive properties of the surface soils present in an assessment unit	Highest:	Non-clay mineral soils are <50% of area	Score = 1
		Moderate:	Non-clay mineral soils are 50-95% of area	Score = 0.5
		Lowest:	Non-clay mineral soils are >95% of area	Score = "0"
Veffectarea2	Areal extent of the assessment unit (as a % of total) that undergoes changes between oxic and anoxic conditions.	Calculation:	% of AU seasonally inundated/100	Enter result of calculation
Vout	The amount of constriction in the surface outflow from the assessment unit	Highest:	No outlet, or severely constricted	Score = 1
		Moderate:	Moderately constricted	Score = 0.5
		Lowest:	Slightly or unconstricted	Score = 0
Index for Removing Nutrients = Total of variable scores x 2.70* divided by score from highest functioning reference standard site (*2.70 is the normalizing factor so that total potential scores for each function will be the same)				

Example 2: Willamette Valley (Oregon) riverine impounding and slope/flats HGM method (Adamus and Field, 2001).

For the Willamette Valley HGM assessment of riverine impounded and slope/flat wetlands (Adamus and Fields, 2001), the following functions were chosen:

- Nitrogen removal
- Sediment stabilization and phosphorus retention
- Water storage and delay
- Thermoregulation (riverine impounded only)
- Amphibian & turtle habitat support
- Anadromous fish habitat support
- Breeding waterbird support
- Invertebrate habitat support
- Primary production
- Resident fish habitat support
- Songbird habitat support
- Support of characteristic vegetation
- Wintering and migratory waterbird support.

The functions performed by each regional HGM subclass and the indicators for each function were then identified by literature reviews and personal experience. Indicators were limited to those that could be determined on a ½-day site visit at any time of year. Protocols for determining or estimating each indicator were tested and revised in the field, and protocols were revised or indicators were eliminated based on field experience.

An example of the indicators and scoring used for the function "Nitrogen Removal" in the Willamette Valley HGM method is shown in Table 6. (Note that this is an example only and that we do not necessarily agree that these particular indicators apply to this function.) Final scoring was based on the choice of either (1) the highest-functioning standard or (2) least-altered standard. In this method, the results from using either type of reference standard were similar. This may have been because almost all the wetlands in the region had been subject to human disturbance at some time in the past, or may have been a result of the specific indicators chosen.

As in the Washington State method, the scores were relative rather than absolute. For example, a relative score of 0.50 for nitrogen removal in a wetland does not mean that the wetland is removing 50% less nitrogen than the reference wetland.

Table 6. Example of scoring for the function "Nitrogen Removal" in riverine impounding wetlands in the Willamette Valley, Oregon (adapted and simplified from Adamus and Field, 2001.).

#	Indicator	Scale
Note: Proceed with assessing this function only if you note hydric soil features which indicate that oxygen deficits are present in at least part of the site, and thus denitrification may occur		
A	Percent of site that is inundated only seasonally	none = 0 1-10 =.1 10-30 =.3 30-60 =.5 60-90 =.7 > 90 = 1.0
B	Difference between biennial high and low predominating water levels: 0) = no change 1) = difference of one class 2) = difference of 2 classes 3) = difference of 3 classes 4) = difference of 4 classes	0) = 0 1) =.3 2) =.5 3) =.8 4) = 1.0
C	Percent of site currently affected by soil compaction (score): 6 = recent, at >90% of site 5 = recent, at 10-90% of site 4 = recent, at 1-10% of site 3 = >5 years ago, >90% of site 2 = >5 years ago, 10-90% of site 1 = >5 years ago, 1-10% of site 0 = none	5/6 =.1 4 =.2 3 =.4 2 =.6 1 =.8 0 = 1.0
D	Percent of site that was constructed from non-hydric soil: 6 = recent, >90% of site 5 = recent, 10-90% of site 4 = recent, 1-10% of site 3 = >5 years ago, >90% of site 2 = >5 years ago, 10-90% of site 1 = >5 years ago, 1-10% of site 0 = none	6 = 0 5 = .1 4 = .2 3 = .3 2 = .4 1 = .5 none = 1.0
E	Number of kinds of dead wood	none = 0 1 =.1 2/3 =.2 4/5 =.3 6/7 =.5 8/9 =.7 10/11 =.9 12 = 1.0
F	Diameter of largest trees (inches)	none = 0 1-12 =.1 13-19 =.25 20-27 =.5 28-44 =.75 45-52 =.9 >52 = 1.0

Table 6, continued. Example of indicators and scoring scales for the function "Nitrogen Removal" for riverine impounding wetlands in the Willamette Valley, Oregon (adapted and simplified from Adamus and Field, 2001.)

#	Indicator	Scale
G	Maximum annual extent (%) of hummocks	N/A
H	Percent of site affected by soil leveling	100 =.1 10-99 =.3 1-10 =.6 0 = 1.0
I	Percent of pools at biennial low water Note: If site is >1 acre, select the condition that predominates in 1-acre subunits of the site	N/A

Function Capacity Score = (avg. of A,B) + C + D + (avg. of E,F) + (avg of G,H) + I

- To calculate a Standardized Function Capacity Score, divide the above Function Capacity Score as indicated below, depending on whether you wish to compare the results to the highest functioning or least-altered condition.

Scale To:	**By:**
Highest Functioning standard	dividing by score from highest functioning reference standard site
Least Altered standard	dividing by score from least altered reference standard

The following is an example of the calculations required for both Functional Capacity Score and Standardized Functional Capacity Score using the adapted Willamette Valley riverine impounding HGM method in Table 6 (Adamus and Field, 2001) for a hypothetical natural riverine impounded wetland.

1. *Determine indicator scores*:

A = 60% of site is seasonally inundated. Score = 0.5
B = Difference of 3 classes between biennial low and high water tables. Score = 0.8
(Average of A + B = 0.6)
C = 0% of site affected by soil compaction. Score = 1
D = 0% of site constructed from non-hydric soil. Score = 1
E = 5 kinds of dead wood. Score = 0.3
F = Largest trees are 21 inches in diameter. Score = 0.5
(Average of E + F = 0.4)
G = 60% hummocks. Score = 0.6
H = 0% of site affected by soil leveling. Score = 1
(Average of G + H = 0.7)
I = 20% of site has pools at biennial low water. Score = 0.2

2. *Calculate the Functional Capacity Score from indicator scores*:

Equation: (avg. of A,B) + C + D + (avg. of E,F) + (avg. of G,H) + I

Equation with values for example wetland: 0.6 + 1 + 1 + 0.4 + 0.7 + 0.2 = **3.9**

3. *Calculate the Standardized Function Capacity Score from the Functional Capacity Score*:

- The Functional Capacity Score for the *highest functioning* reference standard site is 5.2, so the Standardized Function Capacity Score for the example wetland as compared to highest functioning standard = 3.9/5.2 = **0.75**

- The Functional Capacity Score for the *least altered* reference standard site is 5.0, so the Standardized Functional Capacity Score for the example wetland as compared to the least altered standard = 3.9/5.0 = **0.78**

II. Level 3 Overall Sampling Protocol

This section of the report refines and further documents our proposed Level 3 Wetland Assessment Template for the Maryland Department of the Environment (MDE). This template represents the optimum approach for assessing wetland condition and function. However, it is recognized that resource limitations may prevent the full implementation of the recommended protocol. MDE will make the decision on implementation based on available financial and staff resources.

Wetland Site Classification and Reference Area Selection

First, the site designated by MDE for Level 3 assessment will be classified according to the existing MDE (MDE Wetlands and Waterways Program, 2008) draft classification system. Larger sites may have more than one type/class of wetland system(s) within them which will need to be sampled separately. Furthermore, we assume that (A) Level 1+2 assessments have been completed on the site or (B) that requisite data sets, imagery etc. have been obtained and analyzed as necessary.

Secondly, we propose that MDE establish a series of reference sites reflecting a range of disturbance and land use. Thirdly, MDE will select an appropriate and nearby reference site with the same HGM wetland classification or mix of classifications of similar size and which occurs in a similar geologic unit. The site selection may vary according to the purpose of the Level 3 assessment. If it is deemed desirable to compare wetlands to a least disturbed reference standard wetland of the subject type, the reference site should be (1) relatively unaffected by historic nutrient or contaminant inputs and (2) free of significant soil, hydrologic and vegetation disturbances for at least 25 years. However, it may be necessary to include a range of disturbance histories within reference areas. A given reference site may be suitable for comparison to multiple assessment sites.

Sampling Locations

Rationale

The following sample protocol will allow for descriptive and statistical comparisons (1) between Level 3 assessment sites and their designated reference site(s), and (2) to evaluate changes over time within a given Level 3 assessment site. The sample location numbers specified below represent the minimum requirement for simple descriptive and statistical contrasts and for derivation of an approximate water table surface measurements and local groundwater gradients. Once appropriate data sets are acquired to allow development of variance estimators for essential parameters (e.g. vegetation parameters, soil organic matter, litter C:N ratio) additional sampling locations may need to be recommended for the template design based on standard statistical methods that predict minimum sample populations necessary to resolve parameter differences at a given level of probability.

Sample Site Selection

Utilizing rectified imagery and appropriate GIS tools, at least three permanent hydrology, soil, and vegetation sampling locations will be selected using a spatially segregated random sampling approach within each wetland type present. For larger wetland systems (> 10 acres), at least five permanent hydrology/soil sampling locations will be randomly assigned. For very large sites (> 50 acres) at least one permanent location per 10 acres is recommended. Examples of imagery, data layers and a typical sampling plan are given in the *Detail on Methods* section later. Once the random sampling locations (per below) are assigned within each cell, this overall sampling approach becomes a *stratified random sampling* design (Gilbert, 1987).

Before entering the field, a similar number of randomly assigned alternate sampling locations for each wetland or wetland area (e.g. type within a given wetland) will be assigned. To ensure adequate spatial separation of sampling locations (particularly for hydrologic interpretations), all sampling nodes within 50 feet of the boundary of each cell should be excluded from use. Once the pre-assigned point is located in the field, it will be screened against an *a priori* determined set of acceptance/exclusion criteria (e.g. no obvious recent excavations, strong local micro-topographic abnormalities or permanently ponded conditions). If a site fails the acceptance criteria, the next randomly assigned site within that pre-assigned spatial cell will be confirmed and assessed.

Sample Site Establishment and Marking

Each acceptable sample site within each wetland area will be permanently located by driving an 18 inch rebar stake at plot center and by GPS. The rebar stake will be driven to approximately ground level to avoid injury to wildlife and humans, but will be readily sensed by a conventional metal detector if needed. For ease of relocation, a 24" white PVC pipe may be placed over the rebar. At a prescribed distance and azimuth (e.g. 2 m North) from the plot center monument, a piezometer nest and a standard USCOE monitoring well will be installed as described below. This location may be modified to avoid large woody stems or obvious surface irregularities as needed, but any changes in location must be carefully recorded. A baseline soil sampling location will be located along the opposite azimuth (e.g. 2 m South) from the plot center pin or monument. Future soil/litter samples must be taken nearby, but at least 1 m away from this baseline location and all initial and subsequent sample locations must be carefully recorded.

If sedimentation rates will be measured, sediment disks or feldspar marker horizons (Cahoon and Turner, 1989; Kleiss, 1993; Harter and Mitsch, 2003) can also be located near the center pin. Again, these should be located at a prescribed distance and location away from the monument. Sediment traps and feldspar markers should not be situated next to wells or piezometers since these may impede flow. Alternatively, sediment plates or marker horizons may be located on a transect from inflow to outflow (non-tidal) or high marsh to low marsh (tidal) to document differences in sedimentation. In this case, they should be placed a known distance and direction from a PVC rod or other relatively permanent marking device so that they can be relocated. More specific information on these techniques is provided in the *Detail on Methods* section.

The same plot center pin/monument will be used as the center point for all vegetation, wildlife, hydrologic indicator, and biogeochemistry sampling protocols. Vegetation sampling will use non-destructive techniques to all for repeated measures over time (see below).

For tidal sites, the procedure of Cahoon and Guntenspergen (2010) will be utilized to monitor changes in elevation with time. Essentially, this consists of determining trends in elevation relative to sea level by comparing changes in marsh surface elevation with time with the Surface Elevation Table procedure of Cahoon et al. (1995; 2000; 2002) to records of data on local sea level rise and to local tidal range. More specific information on these techniques is provided in the *Detail on Methods* section.

Hydrology Instrumentation and Sampling

One standard USCOE (Sprecher, 2000; see Fig. 1) monitoring well (to 18”) and a minimum of two piezometers will be installed at each plot center location as described above. The minimum piezometer nest (See Fig. 2) will include one piezometer with an open screen at 10 to 12 inches below ground surface and a second piezometer with an open screen at 22 to 24 inches below ground surface. However, if the installer encounters a significantly more compact and/or strongly contrasting textured horizon (e.g. a Btg horizon abruptly under an E horizon) within the upper 12 inches, the depth of the shallow piezometer should be adjusted upwards to place the open screen 1 inch above the presumed stratification. In highly stratified soils or in areas of known epiaquic conditions, it may be necessary to install a third deeper piezometer. Whenever possible, the USCOE well and the piezometers should be fitted with an appropriate automated well + data logger. Otherwise, water levels in the wells should be recorded manually on a monthly basis between May 15 and February 15 and on a weekly basis between February 15 and May 15.

On sites where overbank flooding or significant surface water additions and associated ponding are expected, (a) the well risers should be elevated sufficiently to protect the electronics and (b) the sensor technology employed (e.g. pressure transducer) must be capable of reliably measuring the ponded height above the soil surface. For sites where overbank flooding or other major surface water inputs are presumed, the water level/ponding data acquired will be compared against rainfall data from the nearest available source to establish if any fundamental rainfall x overbank water input relationships exist. This will also allow MDE to differentiate between areas that become periodically ponded due to groundwater discharge or local overland flow additions. For example, ponded wetlands that are supported primarily by groundwater discharge would be expected to exhibit a relatively minor water level response to a rainfall event with a considerable time lag while wetlands receiving local overland flow would show a more pronounced and rapid response.

Ideally, the USCOE well will be also utilized to obtain periodic ground water quality samples as described later. If the data logger technology utilized or other design factors preclude this, then a separate dedicated ground water sampling well should be installed at each location with an open screened increment similar to the standard USCOE design. The surface bore annulus of this well must be sealed with a bentonite pack or concrete collar to prevent surface water ingress.

Note: The well/piezometer array specified above will only be appropriate/feasible on sites where the soil surface is strong enough to support access and well installation and stability. Certain areas with thick organic muck layers, low n-value soils etc. will not receive this monitoring, but may be conducive to monitoring with other types of sensors such as TDR probes with appropriate calibration.

Water Quality Sampling

Water quality samples will be taken quarterly following established and MDE/EPA approved sampling, handling and analytical protocols.

Surface Water

Where significant surface water enters and leaves the site in an established channel, one grab sample from the influent and effluent reach will be collected within 10 m of the established jurisdictional boundary.

Where ponding occurs on the site, one grab sample from a representative area (best professional judgment) will be taken if and when ponding is present.

Water depth and approximate sample point cross-sections or ponding extent will be recorded for each sample location and date.

Ground Water

For sites where best professional judgment indicates that either surface water is not present or will not reliably predict potential water quality impacts and status, two wells be sampled at each site for water quality parameters. If significant lateral ground water gradients are present across the site, these two wells should best represent that gradient. The wells sampled will be either the USCOE well (18” or 45 cm deep) or another water quality sampling well installed to a similar depth. Per established protocols, the well will be purged before sampling and allowed to recover before the appropriate water volume is withdrawn. In certain instances and sites (e.g. mineral flats with a significant hydroperiod or seasonally perched epiaquic sites) groundwater will only be available for sampling within the open screened well depth in the wetter months of the year and the quarterly sampling dates should be adjusted accordingly.

Water Analysis

In the field, before preservation, the sample will be analyzed for temperature, pH, EC, and DO using an appropriate hand-held multi-meter. Water samples will need to be chilled and/or preserved in the field per approved surface- or ground water protocols

In the laboratory, the sample will be analyzed for total-N and total-P.

If a given site has been (or is implicated to be) contaminated by some other constituent (e.g. TPH from road runoff or ammonium-N from animal runoff) then additional samples with attendant preservation and analytical protocols may be required. For example, water samples for metal analysis are typically field-preserved with HNO_3 and may require field filtration for total vs. dissolved analysis. Similarly, samples for TPH/PAH analysis must be taken into approved glass containers and carefully filled to avoid any air-filled headspace in the container. Details on sampling and analytical procedures for many elements and compounds in water can be found in EPA's Clean Water Act Analytical Methods procedures[6], or in the latest edition of Standard Method for the Examination of Water and Wastewater (Eaton et al., 2005).

Soil Description/Sampling and Biogeochemistry Indicators

The baseline soil sampling location is described above. This location should be at least 30 cm away from the root collar of large woody trees, obvious windthrow mound/pit features, decayed tree boles etc.

First, a 1.0 ft^2 (or 0.1 m^2) sampling quadrat will be placed over the primary bulk soil sampling and description location. All fresh (< 1 year old and intact/non-fragmented) litter should be discarded. Next, using a knife and trowel as necessary, the entire litter layer (O horizon) should be carefully separated from above the mineral soil (A horizon) contact and placed into a bag.

Secondly, an intact bulk density sample should be taken from the upper portion (0 to 5.0 cm or 2") of the intact A horizon. Next, a bulk sample of the 0-5 cm depth of the A horizon should be collected and bagged for laboratory analysis.

Sequentially, using a soil spade (sharpshooter), excavate an intact plug of soil to a depth of 25 to 30 cm (10 to 12") and describe it completely for soil morphology (Schoeneberger et al., 2002) including redoximorphic features. Based on this description, determine the presence of any and all Hydric Soil Indicators (NRCS, 2010). In certain instances, it may be necessary to describe the soil to beyond 30 cm via auger or sharpshooter excavation to confirm the nature of the underlying horizons for correct application of NRCS Hydric Soil Indicators. Finally, from the bottom of the sharpshooter excavation (25 to 30 cm) collect another set of bulk density and bulk soil samples for lab analysis. Images and examples of soil litter layer and soil profile description techniques are given in the *Detail on Methods* section later.

The litter and soil samples should be chilled immediately after sampling. In the laboratory, these samples should be analyzed with appropriate EPA/Soil Science Society of America procedures for:

Litter layer:

- Dry weight and % ash content

[6] Available on-line at: http://water.epa.gov/scitech/swguidance/methods/methods_index.cfm

- Total C
- Total N
- Total P

Soil:
- pH
- Bulk density
- Total C
- Total N
- Total P
- KCl Extractable ammonium-N and nitrate-N
- Mehlich I (dilute double acid) Extractable Ca, Mg, K and P
- Oxalate-extractable Fe and Al

Note: Soil parameters above are for 0–5 cm sample. The subsoil (25 to 20 cm) sample should be analyzed for bulk density, pH and Mehlich I nutrients only unless this sample is being used as the "nutrient input reference" in lieu of an appropriate reference area sample.

For subsequent soil observations and sampling over time, the soil sample location should be shifted 1 m away from the baseline location and the direction/azimuth of the location carefully noted and recorded.

Certain sites (e.g. created wetlands, altered/drained wetlands etc.) may not possess soil morphological features or chemical properties that accurately reflect their current biogeochemical condition. At these sites, IRIS tubes (Rabenhorst, 2008) should be installed adjacent to the center plot well/piezometer array to correlate active soil redox conditions with recorded soil water levels. Furthermore, the change in matrix soil chroma and redox feature abundance and location must be carefully quantified over time and compared against the original conditions described in the baseline sampling (Daniels & Whittecar, 2004). On such sites, particularly in recently created wetlands, a significant drop in matrix soil chroma or an increase in abundance of redox concentrations (particularly oxidized rhizospheres) can be taken as evidence that the soil is *developing* hydric conditions.

Details on qualitative and statistical comparisons of soil data sets can be found later in the *Details on Methods* section.

Other Site Conditions

The area immediately around plot center will be evaluated for presence of other primary/secondary USCOE hydrology indicators, local micro-topography such as pit/mound extent and scour channels and fluvial deposition, extent of windthrow etc.

Vegetation

Detailed vegetation surveys will be conducted at each site's primary hydrology/soil sampling location. Additional vegetation sampling locations may be necessary to allow for appropriate statistical analysis. A minimum of two dates, one to reflect late spring and one to represent late summer, are recommended to capture vegetation variation due to seasonal changes (Mueller-Dombois and Ellenberg, 1976, DeBerry and Perry, 2004; Ferner et al., in review).

Note: Care must be taken during all sample protocols (hydrology, soil, vegetation, and wildlife) to not trample vegetation in order to allow re-sampling.

Each vegetation sample plot will be centered on the same plot center pin/monument. The plot will consist of a circle of 11.43 m (37.5 ft) to assure minimum compliance with delineation protocols. Vegetation will be divided into a minimum of four strata:

1. trees (>10 cm dbh, >7 m tall),
2. saplings (<10 cm dbh, >7 m tall or designated as tree in literature),
3. shrubs (<10 cm dbh, <7 m tall or designated as shrub in literature), and
4. herbaceous (including all herbaceous species and woody species <1 m tall).

A fifth strata, vines (defined by literature), can be added if deemed important by the investigator.

At each sampling point (a minimum of five for each site), the following sampling methods will be used:

- Saplings and shrubs greater than 1 m in height will be measured in a 5 m radius plot centered on the sampling point (Spencer et al., 2001). Density will be recorded for each shrub and/or sapling species within the plot. Frequency will be calculated as presence/absence using the density counts (Mueller-Dombois and Ellenberg, 1974).

- Tree data will include the diameter at breast height [DBH at 1.4 m (4.5 ft) high] (an estimate of cover; Mueller-Dombois and Ellenberg, 1974) and density of each tree.

- Density will be recorded for each tree species within the plot. Frequency will be calculated as presence/absence using the density counts (Mueller-Dombois and Ellenberg, 1974).

- Data on herbaceous vegetation will be collected from three randomly placed 1 m^2 quadrats will be established around each sampling point [randomization based on azimuth (360 degrees) and a distance from the sampling point]. Within each of the 1 m^2 quadrats (a minimum of fifteen total per site), areal coverage estimates (using a modified cover class scale, sensu Mueller-Dombois and Ellenberg, 1974), and species density data (measured by a direct count of individuals within one randomly-selected 0.25 m^2 corner of each $1m^2$ quadrat) will be recorded. Plant frequency (presence/absence) will be determined from cover data. Once data has been collected, a species area curve will be constructed from the three quadrats. If the curve does not fit the criteria necessary to indicate adequate sample size (less than 10% slope), two more random samples will be collected. Five sets of quadrat data will be the maximum number due to plot size limitations (Mueller-Dombois and Ellenberg, 1974; Johnson, 2000; McCune and Grace 2002).

- All plants must be identified to species level according to the most appropriate manual (see for example Fernald, 1950; Radford et al. 1968; Wofford, 1989; Gleason and Cronquist, 1991; Weakley, 2002; and the Flora of North America Association, 2002). Nomenclature will follow the Flora of North America Association as cited in the USDA-NRCS (2005). Voucher specimens, if collected, should be deposited in an accessible herbarium. In addition, life history strategy (annual, perennial, and facultative annual) for each herbaceous layer species should be documented in accordance with Reed (1988), Gleason and Cronquist (1991), DeBerry and Perry (2004), and USDA-NRCS (2005).

- For created or restored sites, woody species will be classified in the shrub-sapling layer as either volunteer (i.e., naturally colonizing) or planted. This distinction can be made by; 1) a review of the planting list and/or design on the original blueprints and 2) inspection of trees and shrubs onsite (i.e., those occurring in discernable "rows" and/or those consistent with the original planting plans were more likely to have been planted versus those appearing in random displacement through the site). County and state specimen records should be checked against Maryland Natural Heritage records.

Detail on the data compilation and analysis for vegetation are provided in the *Detail on Methods* section later.

Wildlife Habitat

Avian Assessment

This method is suitable for all wetlands. The avian population will be assessed with a 50 m radius 10 minute single observer point count survey of birds performed at least 2 times per year in breeding season (May-July) during the 4 hours after sunrise (Ralph et al., 1995 as modified by Balcombe et al., 2005) with bird call-back surveys. After the point count, bird-call back surveys for appropriate species will be performed from each point count station (Gibbs and Melvin, 1993; Balcombe et al., 2005) to increase the count of secretive species.

To compare data from separate wetlands, the same number of points and same number of visits must be made to each site. Wetland should preferably be visited on the same day or on consecutive days at the same time of day. If two or more sites are visited on the same day, the order of site visits should be changed during the next visit. The minimum distance between point count stations should be 250 m. Birds should not be surveyed when it is raining, during heavy fog, or when noise from wind-blown vegetation interferes with counting.

Amphibian Assessment

This method is specifically for seasonally inundated depressions and other wetlands that are important amphibian habitat.

Amphibian population will be assessed both qualitatively and quantitatively using the Ohio EPA standard method (Micacchion, 2004), which can also be used to sample macroinvertebrates, if desired. The method involves quantitative sampling with funnel traps along with qualitative dipnet sampling. Wetlands are sampled three times every six weeks between late winter/early spring and late spring/early summer (exact dates depend on regional breeding cycles). The late winter/early spring sample is taken at the beginning of the amphibian breeding cycle, and is used to monitor breeding salamander and early breeding frog populations. A middle spring sample evaluates later breeding frogs and amphibian larvae from early breeders. A late spring/early summer sample is used to evaluate amphibian larvae. The detailed protocol can be found at: http://www.epa.ohio.gov/portals/35/wetlands/Integrated_Wetland_Assessment_Program_Part7_AmphIBI_formatted.pdf

Wildlife Habitat Potential Assessment

We recommend that a general assessment of the wildlife habitat potential of the site be performed in addition to the detailed surveys of birds and/or amphibians described above. Ideally, this assessment would highlight habitat features such as food sources, nesting and breeding areas, and cover without preference for specific wetland types or higher wetland successional stages (personal communication, Denise Clearwater). Some examples of this type of assessment have been developed in Massachusetts[7] (Massachusetts Department of Environmental Protection, 2006) and Oregon[8] (Portland Bureau of Planning, 1986)). These methods would need to be adapted to reflect Maryland's characteristic wildlife species and associated habitat, including Maryland's Species of Greatest Conservation Need.

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[7] Massachusetts wildlife habitat evaluation form available here: http://www.umass.edu/nrec/pdf_files/whe_form_and_instructions.pdf

[8] Oregon wildlife habitat evaluation form available here: http://www.ci.oswego.or.us/plan/Planning_Projects/PP09-0011_Sensitive_Lands_Outreach/Second_Look_Task_Force/HAS-blank.pdf

Methods and Corresponding Functions

Table 7 shows the relationship between indicators, methods, and functions for our proposed Level 3 wetland assessment method, while Table 8 shows the relationship between MDE regulatory functions (personal communication, Denise Clearwater) and the proposed Level 3 wetland assessment method.

Table 7. Indicators, methods, and functions for proposed MDE Level 3 wetland assessment method.

Indicator	Method	Function(s)
Water sources and sinks, hydroperiod	Wells and piezometers Estimate precipitation from nearest weather station.	Maintaining characteristic hydrology Surface and subsurface capture and storage of precipitation, groundwater, and/or surface runoff Floodwater detention (for riparian floodplains)
	Piezometers	Maintaining characteristic hydrology Groundwater discharge and recharge
For tidal wetlands only: Trends in elevation and sedimentation rate relative to sea level	Rod surface elevation table used in combination with marker horizons.	Sustainability of all tidal wetland functions (coastal storm protection, sediment retention, nutrient and carbon cycling and retention, carbon sequestration, etc.)
Soil reducing conditions	Soil description to 30 cm or depth required by NRCS hydric soils protocol IRIS tubes for created wetlands and/or other disturbed sites.	Maintaining characteristic biogeochemistry

Table 7, continued. Indicators, methods, and functions for proposed MDE Level 3 wetland assessment method.

Indicator	Method	Function(s)
Nutrient levels and related factors in wetland water, litter, and soil	Water: Total N and total P at inflow and outflow, in ponded areas, and/or in groundwater Litter layer (O horizon): Dry weight and % ash content, total C, total N, total P Surface soil (0-5 cm): pH, bulk density, total C, total N, total P, KCl-extractable ammonium-N and nitrate-N, Mehlich I (dilute double acid) extractable Ca, Mg, K and P, oxalate-extractable Fe and Al Subsoil (25 to 20 cm): Bulk density, pH and Mehlich I nutrients	Nutrient cycling and removal Off-site water quality impacts
Dissolved or suspended organic carbon	Total organic carbon (TOC) at inflow and outflow	Organic carbon export
Other dissolved or suspended pollutants	As needed (e.g. ammonium-N, total petroleum hydrocarbons, metals)	Removal of pollutants
Soil carbon levels	Litter layer (O horizon): Dry weight and % ash content, total C Surface soil (0-5 cm): Bulk density, total C	Carbon accumulation and sequestration
Sedimentation rate	Sediment plates and/or marker horizons	Removal of sediments
Vegetation assemblage	Detailed vegetation surveys by strata: including dominant species, species richness and evenness, species diversity, rare and endangered species.	Providing habitat for characteristic plant communities, including rare and endangered species Maintaining ecological diversity
Avian population	Point count survey of bird populations with bird call-back surveys	Providing avian habitat Maintaining ecological diversity
Amphibian population	Ohio EPA standard method (Micacchion, 2004)	Providing amphibian habitat Maintaining ecological diversity

Table 8. MDE regulatory functions and the proposed Level 3 method.

Function category	**MDE regulatory function**	**Corresponding Level 3 method (if applicable)**
Biological	Providing habitat or habitat support for plants or wildlife	For plants: Direct measure via detailed vegetation survey by strata For wildlife: 1. Birds: Direct measure via point count/call back survey of birds. 2. Amphibians: direct measure through Ohio EPA standard method. 3. For other species: indirect via wildlife habitat potential assessment.
	Furnishing organic material to the aquatic food webs	TOC at inflow and outflow
Water quality (Biogeochemical)	Filtering or storing sediments, pollutants, and excess nutrients	Sediment plates and/or marker horizons. Total N and P and other analyses of water, litter, surface soil.
	Reducing erosion in streambanks, drainageways, etc.	Not quantified via this template (may be covered by MDE stream assessment protocol).
Hydrologic	Headwater wetland – storing, slowing, or reducing floodwater flow	Well/piezometer data and assessment of local topography.
	Floodwater wetland – storing, slowing, or reducing floodwater flow	Well/piezometer data will be interpreted vs. rainfall data from nearest weather station to estimate depth and length of ponding/storage vs. rainfall events.
	Discharging groundwater	Piezometer data
	Recharging groundwater	Piezometer data
	Storing precipitation (seasonal flat wetland)	Well/piezometer data as compared with precipitation data.
	Storing surface runoff or precipitation (isolated depressional wetland)	Can be estimated by increase in water levels in wells well/piezometers in the wetland during and immediately following a storm minus precipitation data obtained from nearest weather station.
	Coastal storm protection (tidal wetland)	Estimation of wetland sustainability via rod SET and marker horizons.

III. Detail on Methods, Examples and Data Interpretation

In this section, we detail and explain certain aspects of the actual sampling approach and implementation. This section assumes basic knowledge and competence in a number of areas and disciplines including soil and vegetation field sampling and simple statistical analyses.

Site Map GIS Analysis and Sampling Design

The following images illustrate an example of the proposed sampling design in an urban wetland in Prince George's County, MD:

- Figure 3 shows the base image with National Wetlands Inventory (NWI) National Hydrology Dataset (NHD), and Soil Survey Geographic database (SSURGO) layers, with a list of associated soil mapping units.

- Figure 4 shows the proposed wetland assessment area subdivided into five study cells of 5 to 7 acres each.

- Figure 5 shows the wetland study cells with the assessment cells with sampling grid node overlay and acreage.

- Figure 6 shows the wetland assessment cells with five random sample nodes chosen within each cell.

- Figure 7 is the final random sample location map.

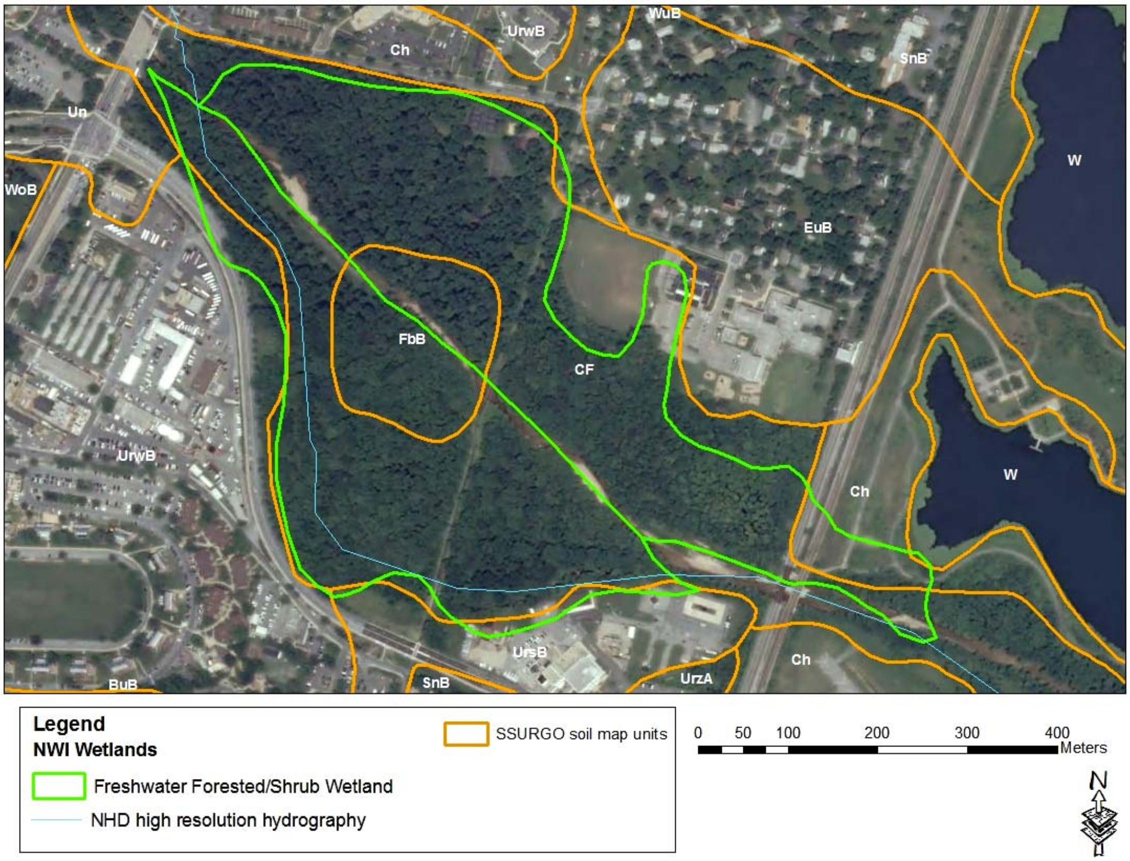

Figure 3. Base image of forested wetland area in Prince George's County with NWI, NHD and SSURGO map units as overlays. Soil map unit legend below. *Note*: the wetland boundary may be based on Level 1 or 2 Assessment data (if available) rather than NWI.

Map Unit Legend

Prince George's County, Maryland

Map symbol	Map unit name
BuB	Beltsville-Urban land complex, 0 to 5 percent slopes
CF	Codorus and Hatboro soils, frequently flooded
Ch	Codorus-Hatboro-Urban land complex, frequently flooded
EuB	Elsinboro-Urban land complex, 0 to 5 percent slopes
FbB	Fallsington-Urban land complex, 0 to 5 percent slopes
SnB	Sassafras-Urban land complex, 0 to 5 percent slopes
Un	Urban land
UrsB	Urban land-Sassafras complex, 0 to 5 percent slopes
UrwB	Urban land-Woodstown complex, 0 to 5 percent slopes
UrzA	Urban land-Zekiah complex, 0 to 2 percent slopes, frequently flooded
W	Water

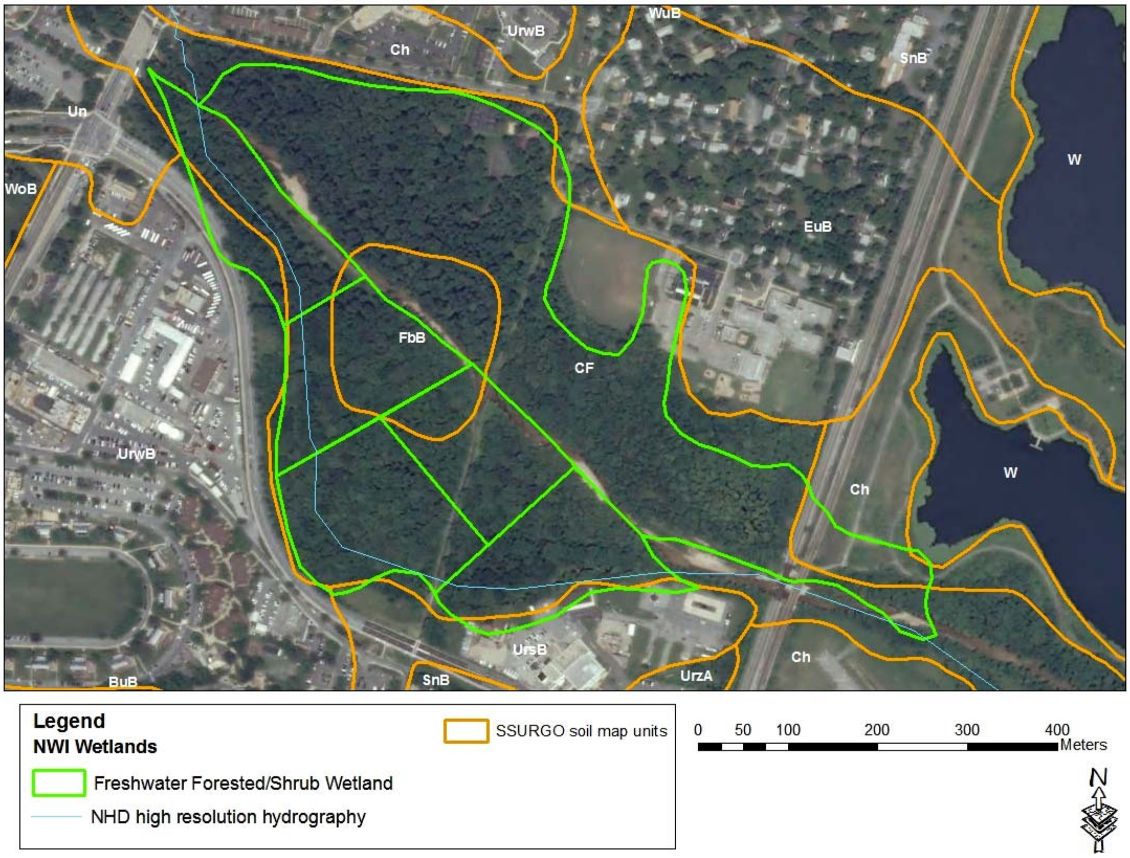

Figure 4. Proposed wetland assessment area subdivided into five study cells of 5 to 7 acres each. *Note*: this subdivision assumes a relatively uniform wetland type across the entire area. If sufficient evidence from Level 1 or 2 assessments indicate different wetland types, the sample cell boundaries and sizes would need to be adjusted to ensure a minimum of three detailed study cells within each wetland type. For larger wetlands, the maximum sample cell size should not exceed 10 acres per type.

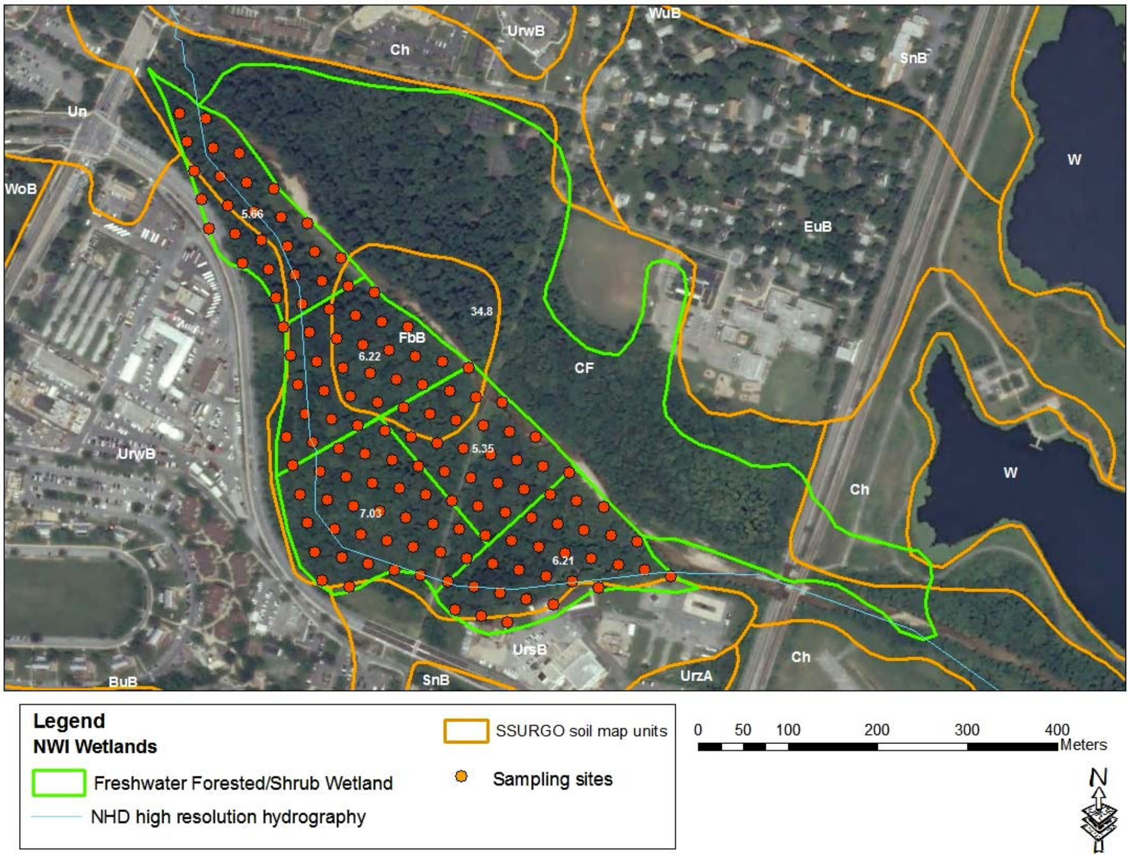

Figure 5. Wetland assessment cells with sampling grid node overlay and acreage shown. The spacing on the grid node can easily be adjusted by the GIS software.

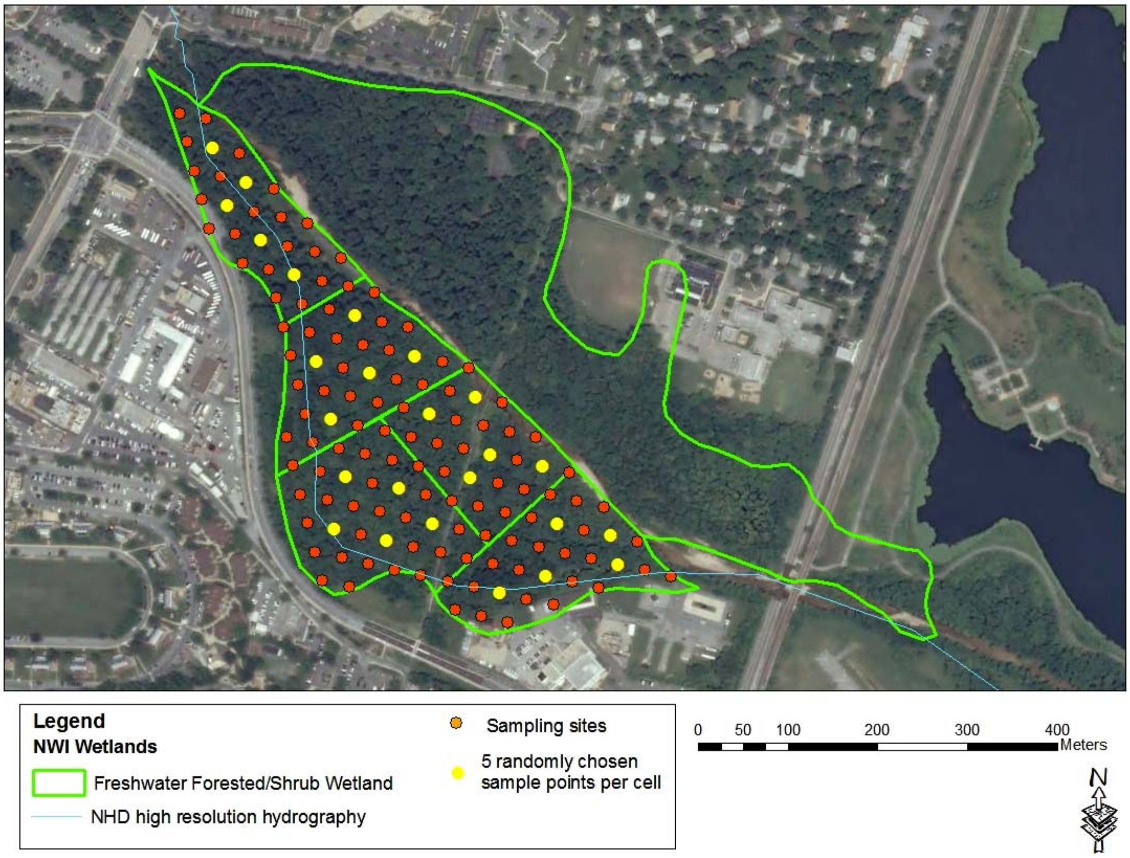

Figure 6. Wetland assessment cells with five random sample nodes chosen within each cell. Sampling nodes within 10 m of a cell boundary are excluded from selection to prevent the potential for two detailed study sites being directly adjacent. This also helps ensure adequate spatial distribution of wells and piezometers for hydrologic interpretations.

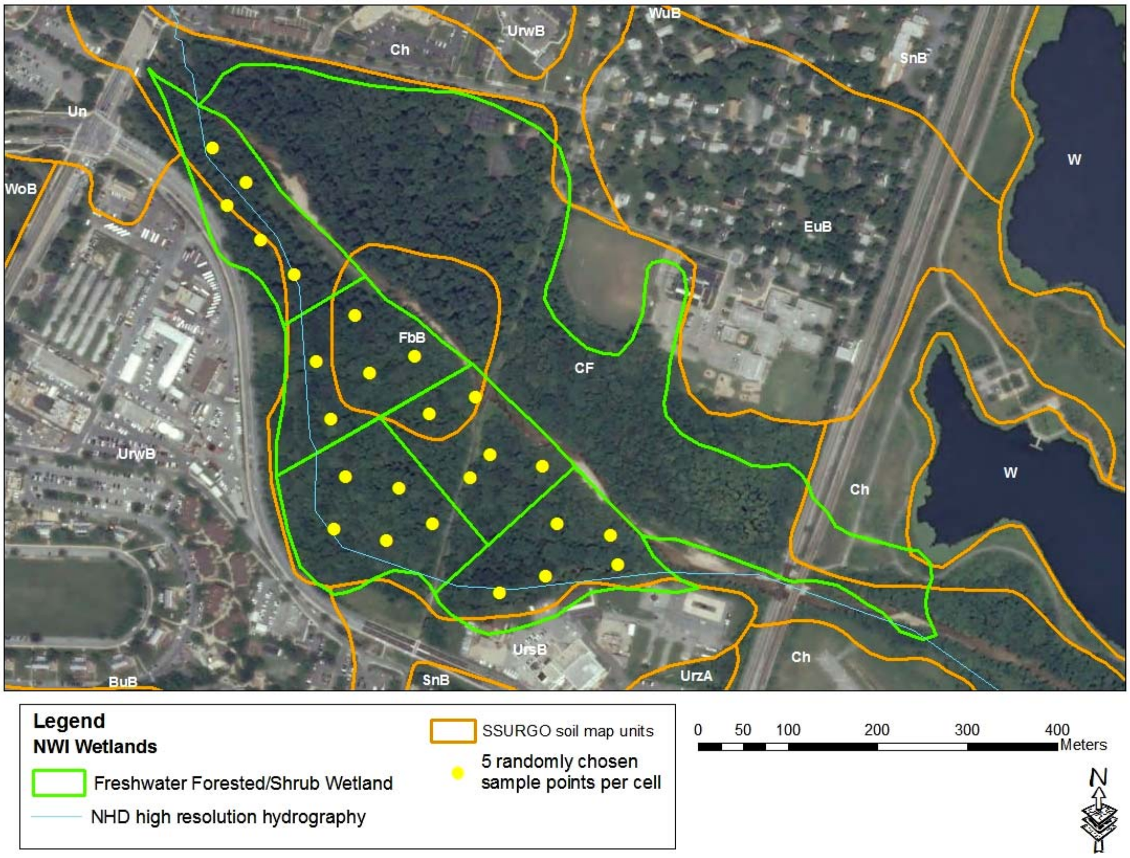

Figure 7. Final random sample location map. Within each cell, the five sampling locations should be randomly assigned numbers 1-5. Next, GPS coordinates are assigned to each. Calculating XY coordinate data for the sample locations within the GIS is straightforward and accurate. ArcGIS provides several ways to calculate the coordinate and transfer these points to the GPS unit for navigation in the field. The field assessment team will proceed to node #1 in each cell and assess its suitability vs. the established *a priori* exclusion criteria. If the location is deemed unsuitable, the team will proceed to node #2, etc. If the location is deemed suitable, the center point is fixed and monumented and all future sampling activities are registered against the plot center position.

Litter and Soil Sampling and Description

Figure 8 and 9 illustrate

- Quadrat sampling of the litter layer.
- An example of an intact soil block removed from a wetland.

Figure 8. Example of 0.1 m^2 sample quadrat in place before litter layer sampling and removal. Using a sharp knife, the litter layer is cut around the inside of the quadrat and then removed by hand down to the mineral soil (A horizon) surface. Where distinctly different layers are present (e.g. Oi vs. Oa[9]) they can be sampled separately. Subsequently, the 0-5 cm layer of mineral soil should be sampled for bulk density and laboratory analysis per the protocol. Finally, the area beneath the quadrat should be excavated with sharpshooter to allow for the deeper (25 cm) soil samples to be collected. With appropriate care, this sampling approach can be combined with the removal of the intact soil plug described in Figure 9. *Note*: Sampling and description of deeper mineral soil layers will be complicated in soils that are ponded such as the one shown here.

[9] Organic horizons are designated Oi (fibric), Oe (hemic), or Oa (sapric) depending on degree of organic matter decomposition. Fibric materials are slightly decomposed, hemic materials are intermediate, and sapric materials are the most highly decomposed.

Figure 9. Intact soil block that has been excavated from a created wetland site per procedures outline in protocol. Note that the surface still needs to be “picked down” with a knife point to better reveal structure, redox feature location/size/abundance and rooting relationships.

Qualitative and Statistical Analysis of Litter and Soil Data

The sampling protocol described here will gather data on litter layer and soil parameters that can be used to determine

- changes over time at a given site and,
- differences in litter and soil parameters between two different sites.

First of all, it is important that identical field sampling and laboratory analytical protocols be utilized at each sampling location and at all times and locations. For example, total soil C can be estimated by a variety of techniques (e.g. loss on ignition, combustion type C:N analyzer, Walkely-Black), all of which can (and usually do) generate substantially different results on the same sample.

A number of important qualitative interpretations can be made at a given sample location at the time of observation such as whether or not soil morphological features meet the minimum requirements for an applicable NRCS Hydric Soil Indicator. Similarly, the thickness and nature of the litter layer(s) and A horizon(s) can often be interpreted with respect to site stability and sedimentation inputs/outputs.

All statistical analyses require some minimum number of observations for their calculation and their *power* to separate real differences in a given parameter (e.g. over time or between sites) increases with the number of observations. While it is beyond the scope of this document to describe the basics of sampling and analytical designs, relevant information can be found in Gilbert (1987) and a very useful tool for quickly calculating the minimum number of sample observations necessary to detect differences between sample populations based on expected variance, desired probability level and other inputs can be found at: http://homepage.usask.ca/~rjb609/NumReps.html. However, this algorithm is specific to normal theory tests (e.g. t-tests) as described below and may over or under predict the number observations necessary for the non-parametric tests described below. For example, environmental data sets frequently contain outliers which lead to this normal theory approach under-predicting the actual number of samples required.

As described earlier, this wetland sampling protocol/template will generate sufficient samples (minimum of 3 to 5 per site/time) to allow for simple statistical comparisons. However, if the sample variance for a given parameter (e.g. total soil P) is high, more sample locations/observation may be necessary or the user may need to accept a lower threshold of probability (e.g. $p \leq 0.10$ vs. 0.05).

For simple statistical comparisons of a given parameter (e.g. total soil C) over time at a given site, it is important to "pair" the data over time by sampling location to reduce the variance estimator and thereby improve the power of the test. The appropriate normal theory test for this would be the "paired t-test". However, normal theory tests such as this are seldom appropriate for small sample sizes ($n < 15$ to 20) and data sets (like soils) that commonly contain outliers. The most appropriate test to employ here is the non-parametric Wilcoxon Sign-Rank Test (Hollander and Wolfe, 1999) which is much more robust for small sample sizes and for data sets with outliers.

In situations where the user wants to compare a given parameter (e.g. plant-available P) between two different wetland sites the most appropriate normal theory test would be the "2-sample t-test." Again, the appropriate application of this contrast is limited when sample numbers are small (pooled $n < 30$) and the more appropriate non-parametric test is the Wilcoxon Rank-Sum Test (Hollander and Wolfe, 1999). All commonly available statistical analysis packages (SAS, Minitab, SigmaStat, etc.) run both the normal theory and non-parametric contrasts.

For comparisons among multiple sites, the non-parametric equivalent of the ANOVA test is known as the Kruskal-Wallis test. Graphical methods such as boxplot displays and probability plots are recommended to use with testing procedures to check assumptions and display patterns. Recent examples where we have applied these statistical tests to wetland soil and vegetation data sets include Bailey et al. (2007) and Fajardo (2006).

Assessing Sedimentation Rates

Sedimentation rates can be assessed with either marker horizons or sediment disks. Marker horizons require no specialized equipment and can be resampled to determine semi-annual or annual sedimentation rates if care is taken to clearly mark and recall previous sampling areas (Cahoon and Turner, 1989; Kleiss, 1993). Marker horizons can also be placed into standing water. Sediment disks allow for easy collection of the deposited sediment for analysis after sediment depth is measured. Marker horizons and sediment disks may be used together if desirable for the goals of the investigation.

Marker Horizons

In the marker horizon method, feldspar clay or another material is spread over the wetland surface in a thin layer in replicated and marked plots (Figure 10). The USGS recommends using feldspar clay for marker horizons in most instances because it is highly visible during resampling. In high energy environments, a heavier material such as silica sand may be better because it does not become resuspended as easily as feldspar clay (Harter and Mitsch, 2003).

Figure 10. Installing a feldspar marker horizon in a forested wetland (photo is from the USGS Surface Elevation Table website at http://www.pwrc.usgs.gov/set/)

The USGS Surface Elevation Table website gives detailed instruction for installation of marker horizons[10]:

1. Select location for marker horizon plot. Typically, three or four replications are used per location. A temporary or permanent sampling platform[11] may be used.
2. Use a 50 cm x 50 cm temporary frame made of wire, PVC or boards to lay out the plot correctly. If the marker horizon plots are to be installed in an area with standing water, a trash can with the bottom removed may be used as a frame to define the plot.
3. Spread the feldspar clay or other marker horizon material in an even layer on the marsh surface or into the trash can. (Feldspar will settle to the bottom of the water in the trashcan in approximately 15 minutes.) If using a trashcan, you will need to wait at least 10-15 minutes (or longer) to allow the feldspar inside to settle to the bottom. If the marker horizon material is spread on top of vegetation, it will need to be knocked off the plants onto the soil surface.
4. Mark the plot corners with PVC or fiberglass stakes, and draw a map showing distance and direction from the center monument or other feature.

[10] http://www.pwrc.usgs.gov/set/installation/markers.html
[11] Instructions for constructing and installing sampling platforms are here: http://www.pwrc.usgs.gov/set/installation/platforms.html

The depth of sedimentation over marker horizons can be resampled by taking cores through the sediments over the marker horizon with thin aluminum soft drink cans with the top and bottom removed (Cahoon and Turner, 1989). Transparent plexiglass tubes may also be used. Alternatively, depth of sedimentation can be measured via cryogenic coring.[12]

Sediment Disks

Sediment disks are 15 cm diameter Plexiglass circles with a 1 cm hole in the middle (Kleiss, 1993). The surface of the disks is sanded to aid in sediment retention.

Disks are installed by (Figure 11):

1. Hammering a 30 cm threaded steel rod into the soil.
2. Placing the disk over the rod.
3. Securing the disk to the rod with a washer and wing nut.

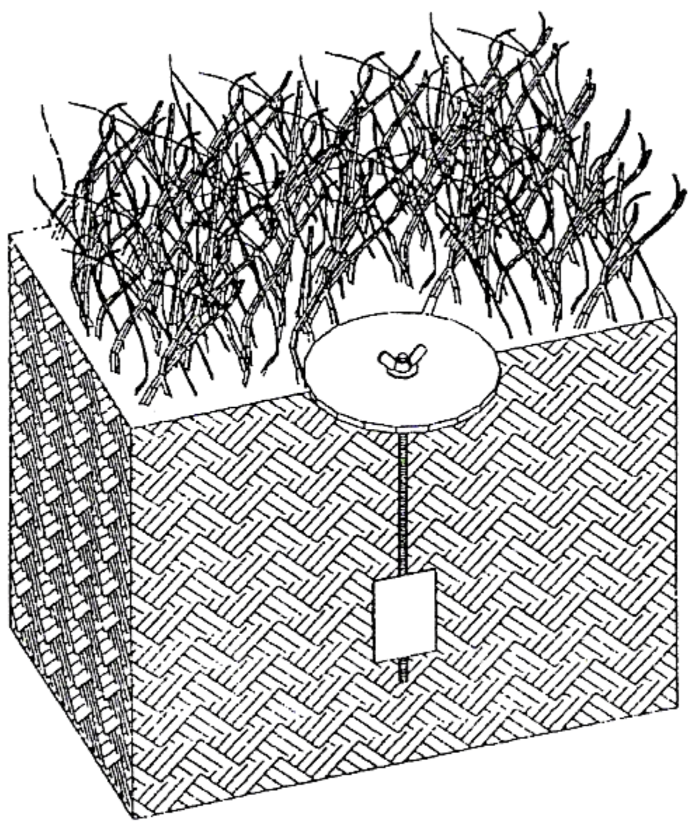

Figure 11. Schematic diagram of plexiglass sedimentation disk (from Kleiss, 1993).

Disks should be placed a known distance and direction from a PVC rod that is securely seated into the soil so they can be relocated. Unglazed ceramic tiles may be used instead of Plexiglass disks but must be fastened securely to the soil surface.

[12] http://www.pwrc.usgs.gov/set/readMarkers.html

If disks are covered with sediment when resampled, the location of the disk is approximated, and a thin metal rod or other tool is used to locate the Plexiglass. The depth of the ground surface above the disk is measured, and accumulated sediments are sampled by cutting around the edge of the disk and placing contents in a sampling bag. The disk should then be elevated onto the new soil surface before re-installation.

Determining Trends in Elevation and Sedimentation Rate in Tidal Wetlands

For tidal sites, the procedure described in Cahoon and Guntenspergen (2010) will monitor changes in wetland elevation with time relative to sea level. This method compares changes in marsh surface elevation with time using the Surface Elevation Table (SET) procedure of Cahoon et al. (1995; 2000; 2002) to records of data on local sea level rise and to local tidal range. The SET procedure allows accurate measurements of sediment elevation of tidal wetland relative to a fixed subsurface data point.

The USGS recommends using the rod SET developed by Cahoon et al. (2002) rather than the original SET described in Cahoon et al. (1995), because it can be attached to benchmarks that are driven to both deeper and shallower depths. This allows measurement of subsidence in different depths of the soil profile. The rod SET is also smaller, lighter, and easier to transport between sites (Figure 12).

Figure 12. Reading the Rod SET at Blackwater National Wildlife Refuge. (Photo from the USGS Surface Elevation Table website at http://www.pwrc.usgs.gov/set/readSET.html.)

Data from the SET can be used in combination with marker horizons to compare sediment accretion rates to elevation change due to shallow subsidence (Figure 13). The marker horizons must be laid out at the same time the baseline SET reading is taken. A typical layout is shown in Figure 14.

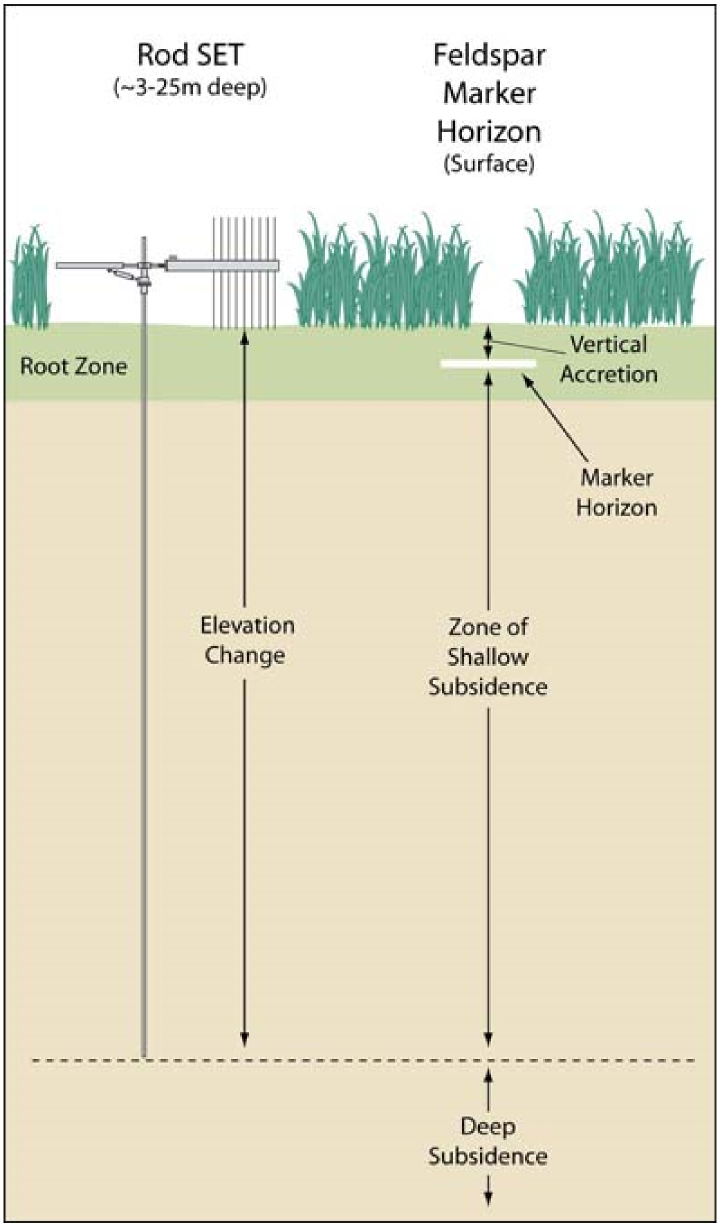

Figure 13. Diagram of the rod SET used with marker horizons (from the USGS Surface Elevation Table website at: http://www.pwrc.usgs.gov/set/theory.html).

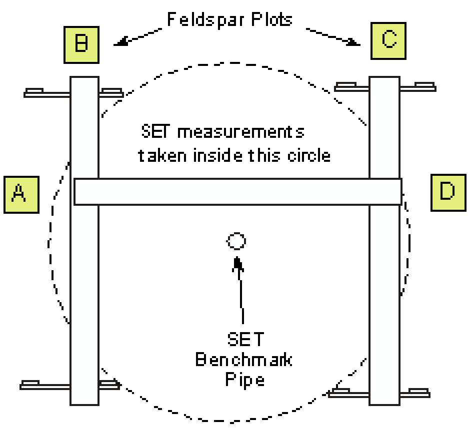

Figure 14. Feldspar plot locations with an SET pipe and permanent platform (from the USGS Surface Elevation Table website at http://www.pwrc.usgs.gov/set/).

Detailed specifications and schematics for the rod Surface Elevation Table and the deep and shallow benchmark are described by Cahoon et al. (2002). Photos, cost estimates, and detailed instructions for installation and use can be found at the USGS Surface Elevation Table website.[13]

[13] Rod SET design: http://www.pwrc.usgs.gov/set/SET/Rod.html
Deep benchmark installation at: http://www.pwrc.usgs.gov/set/installation/InstallROD.html
Shallow benchmark installation at: http://www.pwrc.usgs.gov/set/installation/Installshallow.html
Details on reading the SET at: http://www.pwrc.usgs.gov/set/readSET.html

Vegetation Metrics and Statistical Analyses

The following section details the full range of metrics and statistics by which the vegetation data could be analyzed. Specific statistical analyses will be chosen by MDE as desired.

Vegetation results from procedures such as those outlined below yield a quantitative description and statistical comparison of the vegetation assemblages within and between sites. This includes: 1) vegetation composition (dominant species, species richness and evenness, and species diversity, presence of rare or endangered species), 2) comparison of vegetation assemblages of a site to that of a reference site, 3) the potential for time series analysis of succession (projection of succession in created and restored sites), and 4) measurement of alpha (species), beta (within sites), and gamma diversity (between sites) of Maryland wetlands.

Importance Values

For herbaceous vegetation, cover, density, and frequency data will be converted to relative values and averaged to develop relative Importance Values (IV) by species for each site (Perry and Atkinson, 1997).

For shrubs and saplings, IV is calculated as the average of the relative value of density and frequency. [*Note*: For small sample sizes (i.e., less than fifteen plots per site), frequency (presence/absence) tends to artificially inflate the importance of rare species within the plots, and would therefore was not used in calculating IV's for woody species (personal communication, S. A. Ware, Dept. of Biology, College of William and Mary)]. Overall dominant species are determined by applying the 50:20 rule to mean IV across all 15 sites (Tiner, 1999).

Age Classes (for created/restored wetlands)

For the purposes of drawing ecological distinctions between successional stages of vegetation development, all 15 sites will be grouped into a priori categories based on site age: 1-2 years, 3-5 years, 6-10 years, and five year intervals after the last. The rationale for category divisions is based on a review of several references (Reinartz and Warne, 1993; Noon, 1996; Odland, 1997; Mitsch and Gosselink, 2000; Atkinson and Cairns, 2001; Heaven et al., 2003; DeBerry and Perry 2004; Atkinson et al., 2005). Within each age class, dominant species are calculated as described above.

Similarity and Community Metrics and Statistics

A Sørensen similarity index (SI) or similar-dissimilarity matrix is used to analyze differences in species composition between age classes (Mueller-Dombois and Ellenberg, 1974) using the formula: $2c / (a + b)$, where c is the number of species two age classes have in common, a is the number of species in the first class, and b is the number in the second class. Species composition is further evaluated with Analysis of Similarity (see below). Shannon's Diversity Index (H') (Pielou, 1975) is calculated within each age class and reference area using the algorithm in, or similar to, PC-ORD (McCune and

Mefford, 1999). During sampling, a running mean is calculated on species per sample unit (e.g., plot or quadrat) to evaluate sample adequacy (Mueller-Dombois and Ellenberg, 1974).

Statistical Significance Tests

Data sets can be compared using a variety of parametric and non-parametric tests. Cochran's test is used to measure homogeneity of variance (Cochran, 1941; Fried, 1976). Most vegetation data will indicate homoscedasticity, therefore the homogeneity assumption of parametric statistical tests are violated. Further, because of the type of community data collected, the probability distribution of species at each site is attended by a large number of zeros (i.e., plots in which species are not represented), which produces a positively skewed distribution and violates the assumption of normality (McCune and Mefford, 1999). Therefore, non-parametric methods are normally used to test for significant statistical relationships at the 95% confidence limit ($\alpha = 0.05$). Wilcoxon paired-samples signed-rank test (Wilcoxon test) and Kruskal-Wallis one-way analysis of variance by ranks (Kruskal-Wallis Test) can be performed for comparisons of community variables (species richness, diversity, annual/perennial distribution, planted/volunteer distribution) over site age (time) as a continuous variable (Sheskin, 1997), and a non-parametric version of the Tukey's HSD test can be used to evaluate pairwise comparisons among age classes (also referred to as the Nemenyi-Damico-Wolfe-Dunn test; Hollander and Wolfe, 1999).

Analysis of Similarity (ANOSIM) can be calculated to evaluate species composition differences between age classes (categorical variable). ANOSIM accounts for differences in species composition by analyzing the entire abundance matrix (not just presence-absence by site) (Kindt and Coe, 2005). The benefit of this test is that it provides additional data on similarity that is subject to statistical confidence. Statistical tests can be performed using SysStat, Matlab, PCOrd-5, R, or any comparable statistical computing software. There has been good success in using R as a modeling platform (Kindt and Coe, 2005).

Nonmetric Multidimentional Scaling (NMS) and Canonical Correspondence Analysis (CCA) community ordination is a useful procedure to evaluate environmental variables and vegetation dynamics (ter Braak 1986, McCune and Grace 2002). We (Perry/VIMS et al.) use the CCA algorithm included in PC-ORD version 5 (McCune and Mefford, 1999), which tests significance of eigenvalue computations using Monte Carlo permutations (n=1000) of the existing data set (sensu Ferner et al., in press). Others have found the NMS and CCA programs in R statistics to work as well (Kindt and Coe, 2005), as R is currently a non-menu based software although there are menu-based additions (Heiberger and Neuwirth, 2008; Highland Statistics Ltd., 2010) and R is accessible through some commercial software such as SAS.

Use of Data and Metrics to Validate Level 1 and 2 Assessments

The data sets generated by the proposed Level 3 assessment protocol can be used to confirm and validate Level 1 and 2 assessment outcomes by:

- Using combined soil, hydrologic and vegetation data to reconfirm the wetland type and jurisdictional status.
- Using the data sets to directly confirm and validate any assumptions or predictions made via Level 1 or 2 assessments of the various wetland functions that are associated with the Level 3 assessment results.

However, the functions assessed via the Level 3 method (Table 7) reflect those requested in MDE's proposal for this study but do not correspond exactly to MDE's current regulatory functions (Table 8). For example, one MDE regulatory function is "Filtering or storing sediments, pollutants, and excess nutrients." The proposed Level 3 method measures sedimentation rate, N and P flux and retention (as well as pollutant retention if desired), but these are all determined separately. Since it is possible that a wetland may not perform all these functions at the same level, validation of the "Filtering or storing sediments, pollutants, and excess nutrients" regulatory function would require combining data from several different Level 3 parameters to produce an assessment model. Further input from MDE will be needed to:

- Develop assessment models for using the Level 3 data to validate Level 1 and 2 assessments.
- Develop criteria for evaluating the degree to which a particular wetland is performing a specific function (as described in next section).

Overall Approach for Quantitative Comparisons to Reference Sites

We understand that one overall goal of MDE in applying Level 3 template data and metrics is the development of a "quality ranking system" for wetlands based on a combination of quantified parameters/functions and qualitative attributes. We have agreed to work with MDE (if requested) beyond the period and scope of this project report to develop that framework. Some of the issues and constraints that any such ranking system faces were summarized in the earlier review section on this topic.

Regardless of the form and nature of the final ranking system, the first step will be the determination of an appropriate rationale for selecting reference areas. We also assume and recommend that these reference areas include a range of conditions from relatively undisturbed systems to those with a range of disturbances. Secondly, MDE will need to apply the Level 3 sampling protocol to a wide range of reference areas (stratified by wetland type, region, etc.) to develop a sufficiently robust data set to allow for a reasonable determination of the actual distribution of important parameters such total vs. bioavailable P, sediment accumulation depths, subsoil bulk density, vegetation diversity indices, wildlife habitat parameters, etc. As these data sets are assembled, their parameter distributions will be analyzed via a combination of graphical and statistical techniques to determine whether additional sites or parameter samples are required to adequately specify those distributions.

Once that process is completed, MDE will then be able to compare the properties of interest from a given new assessment site(s) against the reference distributions and make statistical inferences about where a given study site falls in relation to the reference areas. For example, if the surface soil bioavailable P for a given wetland were found to be 20 mg/kg, it could be estimated that it was in the $90^{th} \pm$ SE percentile of similar reference wetlands P levels. Note that this approach uses the reference standard concept of Hruby et al. (1999), in which reference standard sites are those "perform at the highest level for individual functions, regardless of level of alteration," rather than the "least altered" reference standard used in classic HGM assessment models (Brinson and Rheinhardt, 1996).

The next, and more complex, portion of the overall process will be the development of internal quality ranking metrics for each analyzed parameter (e.g. bioavailable P). We propose that this be done via a combination of a review of pertinent literature once a subset of metrics for this purpose is selected combined with detailed statistical analyses of the full data sets from the reference wetlands. For this example (bioavailable P), analysis of the reference site data might reveal important relationships between soil P levels, water quality parameters and vegetation indicators that could then be used to establish relative quality rankings for that parameter.

The concluding aspect of the development of the final ranking system will require making decisions about (a) which and how many parameters/functions to include, (b) relative weightings for each, and (c) other more qualitative factor ratings such as occurrence within urban areas and/or importance of a given wetland for regional wildlife habitat connection. We look forward to working with MDE on the development of this ranking system.

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